

CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(Agents for the United States)

BOMBAY, CALCUTTA, MADRAS: MACMILLAN

TOKYO: MARUZEN COMPANY, LTD

All rights reserved

JOURNAL OF GENETICS

EDITED BY
R. C. PUNNETT, M.A., F.R.S.

Volume XL. 1940



CAMBRIDGE
AT THE UNIVERSITY PRESS
1940

$$\frac{5705-15}{2}$$

PRINTED IN GREAT BRITAIN BY W. LEWIS, M.A.
AT THE UNIVERSITY PRESS, CAMBRIDGE

CONTENTS

Nos. 1 and 2 (May 1940)

	PAGE
H. J. MULLER. An Analysis of the Process of Structural Change in Chromosomes of <i>Drosophila</i> . (With One Text-figure) .	1
M. J. D. WHITE. The Heteropycnosis of Sex Chromosomes and its Interpretation in Terms of Spiral Structure. (With Plates I-III and Nine Text-figures)	67
H. W. HOWARD. The Genetics of <i>Armadillidium vulgare</i> Latr. I. A General Survey of the Problems. (With Plate IV and Five Text-figures)	83
M. B. CRANE. Reproductive Versatility in <i>Rubus</i> . I. Morphology and Inheritance	109
P. T. THOMAS. Reproductive Versatility in <i>Rubus</i> . II. The Chromosomes and Development	119
M. B. CRANE. The Origin of New Forms in <i>Rubus</i> . II. The Loganberry, <i>R. loganobaccus</i> Bailey. (With Plate V and One Text-figure)	129
P. T. THOMAS. The Origin of New Forms in <i>Rubus</i> . III. The Chromosome Constitution of <i>R. loganobaccus</i> Bailey, its Parents and Derivatives. (With Plate VI and Thirteen Text-figures)	141
N. DOBROVOLSKAIA-ZAVADSKAIA. Hereditary and Environmental Factors in the Origin of Different Cancers. (With Seven Text-figures)	157
O. H. FRANKEL. Studies in <i>Hebe</i> . II. The Significance of Male Sterility in the Genetic System. (With Ten Text-figures) .	171
C. D. DARLINGTON and L. LA COUR. Nucleic Acid Starvation of Chromosomes in <i>Trillium</i> . (With Plates VII-IX and Fourteen Text-figures)	185

	PAGE
YASUJI HOSINO. Genetical Studies on the Pattern Types of the Ladybird Beetle, <i>Harmonia axyridis</i> Pallas. (With Twenty-nine Text-figures)	215
K. MATHER and S. B. NORTH. Umbrous: a Case of Dominance Modification in Mice. (With Two Text-figures)	229
A. E. WATKINS and SYDNEY ELLERTON. Variation and Genetics of the Awn in <i>Triticum</i> . (With Plates X-XII and Two Text-figures)	243
J. B. HUTCHINSON. The Application of Genetics to Plant Breeding. I. The Genetic Interpretation of Plant Breeding Problems	271
V. G. PANSE. The Application of Genetics to Plant Breeding. II. The Inheritance of Quantitative Characters and Plant Breeding	283
M. J. D. WHITE. The Origin and Evolution of Multiple Sex-chromosome Mechanisms. (With Six Text-figures)	303
No. 3 (August 1940)	
G. H. BEALE. The Genetics of <i>Verbena</i> ? I. (With Plates XIII-XV)	337
DOROTHY WRINCH. The Fabric Structure of Proteins with special reference to Cytogenetics	359
A. C. FABERGÉ. The Equivalent Effect of X-rays of Different Wave-length on the Chromosomes of <i>Tradescantia</i>	379
G. F. SLEGGs. The Significance of Diploidy and Crossing-over (Theory of Differential Periodicity). (With Five Text-figures)	385
HELEN SCHOCH-BODMER. The Influence of Nutrition upon Pollen Grain Size in <i>Lythrum Salicaria</i> . (With Three Text-figures)	393
THEODORE WHITE. Linkage and Crossing-over in the Human Sex Chromosomes. (With Eleven Text-figures)	403

Contents

vii

	PAGE
J. M. RENDEL. Note on the Inheritance of Yellow Bill Colour in Ducks	439
S. M. SIKKA. Cytogenetics of <i>Brassica</i> Hybrids and Species. (With Plates XVI-XX containing Figs. 1-153, and Three Diagrams in the Text)	441

AN ANALYSIS OF THE PROCESS OF STRUCTURAL CHANGE IN CHROMOSOMES OF *DROSOPHILA*¹

By H. J. MULLER

Institute of Animal Genetics, University of Edinburgh

(With One Text-figure)

CONTENTS

	PAGE
I. Status of the problem	2
II. Effect of varying the conditions of treatment—indepen- dence of effects of individual ionizations within the spermatozoon	7
(a) Lack of effect of temperature	8
(b) Lack of effect of wave-length	9
(c) Lack of effect of intensity and duration of treatment	11
(d) Lack of effect of intermissions in treatment	13
III. The $3/2$ power rule: combination of the effects of individual ionizations after fertilization	14
(a) The frequency-dosage relation for ordinary translocations	14
(b) The relation for gross structural changes involving limited regions	14
(c) The dissociation of the process of structural change into two distinct stages	17
(d) The sufficiency of the breakage-first mechanism in the genesis of the $3/2$ power rule	17
(e) A further test—the rise in the exponent at lower doses	19
IV. Other evidence that breakage precedes union	21
(a) The frequency-dosage relation for losses of chromosomes	21
(b) Chromosome mosaics and biparental rearrangements as evidences that fusion of broken ends may be delayed	25
(c) The frequency relations of multiple exchanges	27
(d) The overlapping of the two stages of structural change in ordinary cells	31
V. The production of minute rearrangements by a spreading of the effect of individual ionizations	35
(a) The linearity of the frequency-dosage curve	35
(b) The delay between breakage and union	39
(c) Bearings on the interpretation of "sensitive volume"	41

¹ This paper is based upon that read on 23 and 29 August 1939 before the Seventh International Congress of Genetics, Edinburgh. As there is room in the present paper only for a statement of the argument as a whole, accompanied by a bare summary of the data of various kinds obtained in this series of experiments, we must reserve for separate papers, to be published jointly with the various workers who participated, all accounts of the details of the individual experiments. These will include descriptions of the experimental calculations—information all of which is needed for estimating the significance of the experiments in question. In addition, it is hoped in most of these papers to have newer data, to supplement those here summarized.

2 *Structural Change in Chromosomes of Drosophila*

	PAGE
VI. Ultra-violet tests: the problem of the relation between structural changes and gene mutations	46
(a) The dubiousness of the distinction between "intra-" and "extra-genic mutations"	46
(b) Ultra-violet as a possible means of discrimination	48
(c) Other evidence regarding chromosome segmentation	51
VII. On the mechanism of radiation necrosis	53
(a) Genetic influences	53
(b) Non-genetic influences	55
Summary	56
Acknowledgements	58
References	59

I. STATUS OF THE PROBLEM

THE work done since the time of the Sixth International Congress of Genetics has greatly strengthened the evidence for the inference then made by both Stadler (1932) and myself (1932) that virtually all viable structural changes in chromosomes—with the exception of some terminal deficiencies in plants, to be discussed later—involve an exchange of chromonemal connexions, as by a kind of heterologous crossing-over: that is, by obligatory fusion at points of breakage and only at such points, each broken end uniting with only one other. This requires at least two points of breakage for each surviving structural change (with the exception above noted). Moreover, the reconstructed chromosome cannot continue to be transported properly throughout the cycles of mitosis and meiosis unless it happens to be monocentric and—in *Drosophila* at least—ditelic, one centromere and two telomeres being necessary and permanent organelles of each *Drosophila* chromosome (Painter & Muller, 1929; Muller, 1938; Muller *et al.* 1937*a*; Belgovsky & Muller, 1937; Raffel, 1938; Belgovsky, 1938).

All kinds of structural changes—translocations of varied kinds, inversions, shifts, deletions, and combinations of any of these—are comprised under these rules, the type of structural change occurring in a given case being merely the result of the topographical accidents of where the breakage occurred and which broken ends chanced to fuse together.¹ This is not to say, however, that the changes are completely random in the sense that all parts are equally subject to breakage and exchange. On the contrary, "heterochromatic" regions—no matter whether or not they include a centromere or telomere—and regions near them are known to be far more subject to breakage and exchange, length for length of the

¹ For diagrams of all types of structural changes capable of surviving indefinitely, derived from two or three breaks, see Muller (1940, Fig. 1).

chromonema, than ordinary regions¹ (Muller & Prokofyeva, 1935; Muller *et al.* 1937 *a*; Belgovsky & Muller, 1937; Belgovsky, 1938).

Despite the establishment of these principles concerning the products of structural change, no general agreement has yet been reached among students of the subject concerning the mechanism whereby these structural changes are brought about. As was pointed out by the author some 10 years ago (Painter & Muller, 1929), two opposite views of the mechanism were conceivable: either that breakage of the chromonemas occurred first—presumably independently in the different places—to be followed by fusion as a later consequence, or that the process of breakage was dependent on the prior approximation and perhaps fusion of the chromonemas—the different breaks concerned being in this case results of a common initiating process. Intermediates between these two extremes were of course conceivable also. The second, or so-called “contact” mechanism, was independently proposed by Serebrovsky (1929) and advocated by him as a general scheme to include all mutations—even “gene mutations”, which he postulated to be minute structural changes. Dubinin and his co-workers have since 1930 championed this view, at least so far as the gross changes are concerned (Dubinin & Khvostova, 1935; Khvostova & Gavrilova, 1935, 1938; Heptner & Demidova, 1936; Kirssanow, 1937; Demidova, 1937). On the other hand, Levitsky and his co-workers (Levitsky & Araratian, 1931; Levitsky & Sizova, 1935), Stadler in his brilliant and prophetic paper before the Sixth Congress (1932), and Mather and the late L. H. A. Stone (1933) have defended the “breakage-first” possibility.

Evidence to decide between the two major possibilities of structural change might (as explained in my paper at the same Congress, 1932) be provided by a study of the frequency of structural changes in relation to dosage of radiation, as well as by an analysis of whether or not multiple or only two-by-two exchanges occur. On the contact view, a linear frequency-dosage relation might well obtain, for in this case individual ionizations might cause the heterologous exchanges of gene connexions, whereas on the breakage-first view the relation should not be so simple, since any rearrangement would result from the coincidence of at least two breaks, each independently caused by the radiation. As data then

¹ As Prokofyeva-Belgovskaya & Khvostova (1939) have found, this applies, though in correspondingly lesser degree, to the less extremely heterochromatic regions (perhaps derived from relatively recent duplications) that they have shown to exist in various interstitial locations (Prokofyeva-Belgovskaya, 1937*b*, 1938). More recently, Kaufmann (1939) has independently obtained evidence to the same effect.

4 *Structural Change in Chromosomes of Drosophila*

existing on this question (Oliver, 1932) seemed—deceptively, as we now know—to show the linear relation, I accepted the contact hypothesis at that time, only to discard it as a general explanation soon afterwards, on the basis of work done on gross deletions in collaboration with Margaret Vogt and the late Lonta Koerner in 1933, which was substantiated by that done on translocations by Belgovsky in 1934 and 1935, and which had in fact been foreshadowed by earlier work of Oliver's (1930), to which insufficient attention had been paid. For our experiments showed that the frequency of gross rearrangements, deletions, translocations, etc., varies exponentially with the dosage, a fact which we took to mean that at least a part of the structural changes, perhaps all, are probably caused by independent breaks, occurring before fusion. The exponent turned out to be approximately $3/2$, however, instead of the square, in all our experiments, and for a time the interpretation of this intermediate result seemed uncertain.

As set forth at the meeting at which these $3/2$ power results were first presented,¹ we also regarded the then unexplained observations of McClintock (1932) on the variation in size of ring chromosomes in maize as furnishing implicit evidence, from a quite different angle, that prior breakage, even when mechanically produced, would in fact be followed by fusion of the broken ends. In our considerations of the matter the sequence of events which this interpretation involves—(1) uncompensated twisting of the chromonema (involving a half or full revolution at one point, in relation to the succeeding point), followed by (2) chromosome division, with resultant formation of dicentric and/or interlocked rings, then (3) breakage of the latter and (4) fusion of the broken ends to form two new rings, of aberrant size—arose as an inference from the earlier findings of Navashin (1930) on the interlocking of chromatids in a ring chromosome in *Crepis*, when regarded in connexion with the size variation of the ring in maize. In the meantime, the remarkable cytogenetic work of McClintock (1938 *a, b*, 1939) on ring chromosomes in

¹ These results on the frequency-dosage relation, and their possible interpretations, were first presented by the author and Belgovsky at a public meeting at the Institute of Genetics, Moscow, 1935, and were communicated privately to British and American colleagues at the same time. They are restated and discussed in the references to this meeting made in publications of Dubinin & Khvostova (1935), and of Kirssanow (1937). They have also been reported by the author in publications of 1936, 1937, and 1938. The data have been given in the following publications: Belgovsky (1937, on translocations), Muller (1938, on deletions). Similar experiments on inversions, carried out at the author's suggestion by Berg, Panshin and Borisoff in 1935 (unpublished), have given confirmatory results, as stated in some of the papers above cited.

maize has led to a direct and convincing demonstration of this sequence of events.

Nevertheless, attempts which we made, during the same period, to obtain evidence of this kind by utilization of the ring *X*-chromosomes of *Drosophila* gave only negative results. For, although these chromosomes did have a tendency to be lost, this proved to be confined to early cleavage stages, and the rings did not tend to give rise to an exceptionally high number of interstitial deficiencies as a result of either their mitotic or meiotic divisions (Bhattacharya and the author, unpublished). This must be because of lack of sufficiently effective twisting of the chromonemas before their division in *Drosophila*, and because the chromosomes forming bridges at meiosis either fail to break or, if broken, fail to reach the egg nucleus (even where all four chromatids have crossed over and formed bridges). Hence the results on *Drosophila* rings, unlike those in maize, could not be used in further elucidation of the mechanism of structural change.

To return to our dosage experiments, the question immediately arose in connexion with our results: What could be the explanation of the intermediate value, between 1 and 2, of our exponent relating frequency of gross structural changes to dosage? One possible interpretation of it was that the "primary events", whether breakages or fusions, were not caused by single ionizations at all, and hence were not proportional in their frequency to the dose. In that case, the frequency-dosage relation observed would not in itself provide any decision between the two mechanisms in question. A second possible interpretation was that a part of the structural changes resulted from single ionizations (as on the contact hypothesis), the frequency of these cases being simply proportional to the dose, while others resulted from two or more ionizations, their frequency varying as the square of the dose; the intermediate result would on that view be an effect of the mixture of the two classes of cases. We also considered, thirdly, the possibility that all the rearrangements were produced as combinations of independent breaks, but that the expected dependence of their frequency upon (nearly) the square of the dose was masked by a differential viability of the recombinational chromosomes formed at different doses. Haldane, in consultation concerning this matter, carried out calculations which substantiated the latter idea (in a personal communication, 1935), and it was suggested independently by Stadler (1936) and recently worked out in some detail by Catcheside (1938 *a, b*) (*vide infra*). Assumptions of random reunion were, however, involved that for a time made such calculations seem in-

secure. Moreover, as above noted, it had still to be proved that the frequency of the "primary events" themselves was, as here assumed, proportional to the dosage, and that these "primary events" were the breakages.

To add to these uncertainties, it was soon afterwards reported by the co-workers of Dubinin (Khvostova & Gavrilova, 1935, 1938; Heptner & Demidova, 1936; Demidova, 1937) that some chromosome rearrangements studied by them—(1) translocations of the fourth chromosome giving a "cubitus interruptus" position effect, (2) rearrangements giving mottled alleles of white, (3) reversions of Plum, and (4) gross deletions of the X-chromosome—showed a simple linear frequency-dosage relation, and Dubinin and his group have thus continued to espouse the contact hypothesis, although later (1937) admitting that translocations of the major autosomes, taken *en masse*, followed the $3/2$ power rule, as we had found. Marshak (1935, 1936, 1937, 1938, 1939 *a, b, c, d*) also has reported the finding of proportionality between cytologically detected chromosome rearrangements and dosage, mainly in plant material, and he too adopts the contact interpretation (assuming the fusion to be made possible by a decharging action of the effective electron).

Because of the above disagreements and doubts, we decided some two years ago (1937) to broaden our attack on the whole problem, and, in conjunction with a considerable group of co-workers at the Institute of Animal Genetics, began the series of studies reported in the present paper.

In the meantime (1938 and 1939), several other series of data on the subject have been reported. The first two of these are by Catchside (1938 *a, b*), and are accompanied by the calculations above-mentioned. Of these, the first (dealing with *Drosophila*) has been regarded by Catchside as not inconsistent with the linear frequency-dosage relation to be expected on the contact view; the second comprises data on plant material dealing with the frequency relations of rearrangements involving double, triple, quadruple, etc., breaks, and he regards these data also as not being out of harmony with the contact view. Similarly, Buzzati-Traverso (1939), writing in the *Drosophila* Information Service, has recently reported finding a linear frequency-dosage relation for *Drosophila* translocations. And Bauer *et al.* (1938) have presented data which according to them do not depart significantly from linearity except probably at one point (a departure which they think might possibly have resulted from "some uncontrolled experimental factor"). To be sure, they describe their results, despite this circumstance, in terms of the breakage view, but their work concerns itself more with the question of

the relative positions of the exchanges than with that of their frequency at different doses, or of a decision between the two alternative mechanisms, and this aspect of the matter is not stressed by them.¹

On the other hand, Sax (1938), in his recent work on structural changes in *Tradescantia* observed cytologically soon after treatment, has independently arrived at the $3/2$ power rule, and interprets his results as furnishing decisive evidence in favour of the application of the breakage-first mechanism in this material. As a matter of fact, his $3/2$ power rule represents something different from ours, the mathematical agreement being largely a coincidence. Nevertheless, as we shall see later, his findings, as well as the parallel ones of Fabergé (1939) (see pp. 32-4) and ours do lead to the same essential conclusions, although by different routes. Moreover, as we have seen, the same may be said of the recent studies of McClintock (1938 *a, b*, 1939).

II. EFFECT OF VARYING THE CONDITIONS OF TREATMENT—INDEPENDENCE OF EFFECTS OF INDIVIDUAL IONIZATIONS WITHIN THE SPERMATOZOON

As the disputed $3/2$ power rule, on the face of it, suggested that the action of the radiation may be of a very indirect nature, in which case the results could not be taken as clear evidence for either of the proposed mechanisms as against the other, it became the more desirable, before deciding definitively between them, to extend the investigation, so as to include a study of other conditions than total dosage that might affect the results. When we began our work it was surprising that, as yet, no such studies had been reported: on the effects of variations in wave length, intensity, timing of irradiation, etc., on the production of structural changes,² such as had been carried out so abundantly and with such definitive results in the case of gene mutations by Timofeeff-Ressovsky and others, although the findings along such lines would necessarily be basic to any interpretations regarding the mechanism involved in the case of structural changes, just as they have been in the case of gene mutations.

In judging of the meaning of results of this kind concerning structural

¹ But in an abstract of a paper submitted to the Seventh Congress by Bauer (1939), who left before the time for his address, this author now states that he has obtained evidence that the frequency-dosage relation, as observed in the salivary glands of *Drosophila* larvae derived from treated fathers, is "between a two-hit and a three-hit curve". If this means that the exponent in question is between 2 and 3 it is higher than was to be expected on either the contact or the breakage-first interpretation, although the latter is regarded by Bauer as furnishing the explanation of it (see p. 24).

² Except for some cytological work of Marshak (1937), involving wave-length studies, which reached our hands when the present work was under way.

8 *Structural Change in Chromosomes of Drosophila*

changes, it will be a useful simplification and will tend to avoid confusion if we first consider them quite apart from the results above referred to concerning the frequency-dosage relation found when the total dosage is altered, and only later bring the latter facts into the picture.

(a) *Lack of effect of temperature*

In the first place we may present the results obtained in our experiments carried out with Dr Makhijani, in which the temperature obtaining at the time of irradiation with X-rays (applied to adult males) was varied from 5 to 37° C. These are summarized in Table I. Here it will be seen

TABLE I
Lack of effect of temperature (5° C. v. 37° C.) *
during X-raying (dose ca. 2000 r.)

Series no.	Tests for lethals in X				Tests for translocations of II with III			
	No. of cultures	No. of lethals	% of lethals	Diff. (in %) and S.E.	No. of cultures	No. of translocations	% of translocations	Diff. (in %) and S.E.
1 Cold	1018	63	6.2	+0.3 ± 1.1	384	11	2.9	+0.1 ± 1.1
Warm	748	44	5.9		398	12	3.0	
2 Cold	848	40	4.8	-0.7 ± 1.1	848	24	2.8	+0.0 ± 0.8
Warm	678	38	5.6		678	19	2.8	
3 Cold	592	31	5.2	-2.6 ± 1.4	592	17	2.9	-0.8 ± 1.0
Warm	610	48	7.9		610	13	2.1	
4 Cold	174	7	4.0	+1.4 ± 2.0	174	1	0.6	-0.1 ± 1.0
Warm	152	4	2.6		152	1	0.7	
5 Cold	764	54	7.1	+0.2 ± 1.4	0	—	—	—
Warm	640	44	6.9		0	—	—	
Combined differences:*				-0.5 ± 0.6	-0.2 ± 0.5			

* The combined difference was obtained by subtracting the weighted average percentage of all series for the warm temperature from that for the cold temperature. The weight (w) by which each individual percentage was multiplied in obtaining this average was taken as the harmonic mean of the numbers of cultures examined in that series at both temperatures (i.e. $w = 2/(1/n_1 + 1/n_2)$, where n_1 and n_2 are the numbers of cultures at the cold and the warm temperatures, respectively, in the given series). We have calculated that the standard error of the combined difference so obtained has the following value:

$$\frac{\sqrt{[\Sigma (2wpg)]}}{\Sigma w}$$

Here p represents the proportion of lethals found in a given series at both temperatures taken together, q is the proportion of non-lethal cultures in that series as a whole, and w is the weight.

that the frequency of the translocations produced in spermatozoa, like that of sex-linked lethal mutations,¹ remains sensibly unaffected by this

¹ Since the above was presented, a paper has been received from Timofeeff-Ressovsky & Zimmer (1939), reporting recent experiments showing the ineffectiveness of temperature in modifying the frequency of sex-linked lethals induced by X-rays in *Drosophila* spermatozoa.

considerable temperature difference. Corroboratory evidence to the same effect is to be found in Table IX on p. 35. Previous reports of an influence of temperature are probably to be ascribed mainly to the difficulties in the way of securing a technique in which the secondary radiation is the same in the two contrasted series, and to the inclusion of results from male germ cells treated in an immature (pre-spermatozoon) stage (since in such cells, as explained in § IV (*d*), an influence of temperature on the final result would be expected, even though it did not affect appreciably the immediate action of the radiation on the chromosomes).

The ineffectiveness of temperature at irradiation indicates that the physical effects of the rays—quantum absorptions or ionizations, which are known to be virtually unaffected by temperature—ordinarily constitute the limiting factors in the determination of whether or not any given structural change shall be produced. As the events following these original physical changes are thus seen to move inevitably towards their conclusions within the sperm, regardless of temperature, it is unlikely that they act by initiating diffuse chemical changes whose products in turn, by their concentration, secondarily influence the probability that structural changes in the sperm will occur. For the chemical reactions dependent on these products would in this case be of a labile nature, and so their occurrence would be likely to be affected by temperature. Thus the negative temperature result argues for the translocation-determining effects within the sperm probably being localized effects, representative either of the originally absorbed quanta of incident radiation or of the individual ionizations formed in the electron paths. The present temperature result will also prove to have a bearing on some of our other experiments, by showing that the unusual temperatures that formed a part of the technique in them did not introduce a disturbing factor into the results.

(b) *Lack of effect of wave-length*

The decision whether it is the quanta of radiation originally absorbed, or the ionizations subsequently resulting from these quanta, which determine the production of the structural changes, can be made by observing the effect of varying the wave-length of the radiation. Gamma rays of radium, having much shorter wave-length, give far larger absorbed quanta than X-rays, and so it takes far fewer quanta from γ -rays to produce the same total number of ionizations. Thus, if the structural changes depend on the quanta originally absorbed but not on the ionizations finally produced, γ -rays inducing a given total number of

10 *Structural Change in Chromosomes of Drosophila*

ionizations, that is, having a given "dosage", should cause far fewer structural changes than X-rays of the same "dosage", whereas they should induce the same frequency of structural changes as the X-rays if these changes result not from the original quanta in themselves but from the final ionizations.

When, with this question in view, the results on translocations from our above experiments with X-rays at different temperatures are compared with those from experiments with γ -rays of similar dosage, carried out with Dr Ray-chaudhuri, it is seen that the frequency of structural changes discloses no influence of the enormous difference in wave-length between γ -rays and 50 kV. X-rays (see Table II, last two lines). The temperature differences in the experiment may legitimately be neglected, as we have seen above, and that the differences in time and intensity of

TABLE II

*Comparison of X- and γ -rays in production of translocations
(of type II-III) in spermatozoa*

Experiment	No. of cultures	No. of trans- locations	% of trans- locations	% lethals in X
X-rays, 1500 r. (combined series from timing experiment)	2223	56	2.5 ± 0.4	5.9 ± 0.7
Above results calculated for 2000 r. (on $3/2$ power rule for translocations)	—	—	3.8 ± 0.5	7.9 ± 0.9
X-rays, ca. 2000 r. (combined series from temperature experiment)	3836	98	2.9 ± 0.3	6.0 ± 0.2
γ -rays, 2000 r. (combined series, all at 8°C.)	942	36	3.8 ± 0.6	6.1 ± 0.6

treatment may also be neglected will be shown presently. Likewise, the combined results from our X-ray experiments in which the timing was varied (to be reported in greater detail later), which utilized a total dosage of 1500 r., showed no effect attributable to wave-length (see upper two lines of Table II). The differences in frequency of translocations found between this and the γ -ray and other X-ray experiments are merely those that might have been expected in view of the comparatively small difference in total dosage, on the basis of the $3/2$ power frequency-dosage relation, regardless of the wave-length. This being the case, it may be concluded that the structural changes result, somehow, from the final ionizations, and not directly from the original quantum absorptions. At the same time, the great differences between γ - and X-rays in regard to the spacing of the ionizations produced at any given moment are also seen to be without influence here.

(c) Lack of effect of intensity and duration of treatment

Radiation intensity also was varied very widely in our experiments, as seen in Table III. In the experiment with γ -rays the flies (impregnated females, kept at 8° C. during treatment) were divided into two lots, one lot (row 3 in Table III) receiving the total dose of 2000 r. in a period of 40 hr., that is, at a rate of 0.8 r./min., and the other lot (row 4 in the table) having the same total dose spread out continuously over 30 days, that is, at $\frac{1}{20}$ r./min., or only $\frac{1}{16}$ the intensity of the other lot. The 0.8 r./min. lot yielded sixteen translocations between paternal second and third chromosomes in a total of 473 cultures, and the 0.05 r./min. lot twenty in a total of 469, i.e. sensibly the same frequency. In the previously mentioned X-rayed lots, which, as we have just seen, also gave

TABLE III

Independence of translocation production in spermatozoa from the intensity-time relation of irradiation (Bunsen-Roscoe Rule)

r./min.	Total dose in r.	No. of cultures	No. of trans-locations (II-III)	% trans-locations calculated for 2000 r.	Type of rays	Conditions of treatment
250	ca. 2000	3836	98	2.9 \pm 0.3	X	Combined series from temperature experiment
100	1500	2223	56	3.8 \pm 0.5	X	Combined series from timing experiment
0.8	2000	473	16	3.4 \pm 0.9	γ	8° C.
0.05	2000	469	20	4.2 \pm 1.0	γ	8° C.

frequencies agreeing with this when allowance is made for the total dosage of a part of them having been somewhat below 2000 r., the intensity was approximately 100 r./min. in the timing experiments and 250 r./min. in the temperature experiments, so that the whole range of intensity-time variation in the experiments with γ - and X-rays, combined, represents a factorial difference of about 5000 times, or nearly four orders of magnitude. In terms of the average number of ion-pairs induced in each spermatozoon the range is from about 900 ion-pairs per minute down to about 1 ion-pair in $5\frac{1}{2}$ min., without any perceptible difference in the effect. Throughout this range, then, the Bunsen-Roscoe law holds for structural changes just as it does for gene mutations.¹

¹ For gene mutations, the work of Timofeeff-Ressovsky and others has shown the Bunsen-Roscoe law to hold from about 300 r. down to 1 r./min. (Timofeeff-Ressovsky & Zimmer, 1935a), while experiments carried out by Ray-chaudhuri in collaboration with the author, in parallel with the above translocation work, now nearly doubles the range for gene mutations, by extending it from 1 r. down to 0.01 r./min. (see Ray-chaudhuri, 1939).

12 *Structural Change in Chromosomes of Drosophila*

Of course 1 ion-pair in $5\frac{1}{2}$ min. represents an average for the low-intensity treatment, whereas the ions really come in spurts, at the times when the individual quanta are absorbed, arranged along an electron track. But when we consider that in the very case in which there was the lowest intensity of radiation γ -rays were used, as a result of which these ions are spread very far apart (at distances of the order of microns) along the electron tracks, we see that the ions cannot really be necessary in groups at any given moment, in order to produce their effects on the chromosomes. That is, we find that, within the limits of our experiments, for any given total dose, no matter how isolated in space and time or how crowded in its association with its fellow ions each given ion is, it has the same likelihood of being instrumental in the production of a structural change.

Hence, whatever may be the nature of those effects, capable of leading to observed structural changes, which are produced within the spermatozoa by the ionizations—whether those effects be the final structural changes themselves or some other changes, antecedent to the structural changes as we ordinarily observe them—in any case, those effects within the spermatozoa must be conceded to be produced by *individual* “fortunate” ionizations, and those effects must persist and accumulate quite independently of one another within the spermatozoa so long as the latter are undergoing treatment, even throughout the course of 1 month. If now we should suppose that these individual ion effects were not the structural changes themselves, but that they had somehow to co-operate, interact, or enter into combination with one another in, secondarily, producing the structural changes, we should have to conclude that they could not enter into these interactions effectively until after the whole of the treatment had been given and all of them had been produced. For the number of such interactions possible, and hence their likelihood of occurrence, would necessarily be a function (an exponential one) of the concentration of the individual ion-effects present, and this concentration could never attain the same maximum in the treatments of different intensity-time relations, unless the individual ions or ion-effects persisted and accumulated over an indefinitely long time, namely, so long as the treatment was being given. No interaction or combination of effects, then, occurs during the time of treatment.

In this connexion it should be noted that Catcheside, in his paper before the Seventh Congress (1939), has presented data, obtained in 1939, which give independent evidence of the lack of effect of the time-intensity factor on the production of gross rearrangements. In his experiments the

duration of treatment varied from $\frac{1}{4}$ hr. to somewhat over 5 hr. (a factorial difference of over $20\times$), and the changes were detected by examination of the salivary glands of *Drosophila* larvae derived from treated fathers. Catchside likewise has inferred that the effects of the individual ions in the sperm must be independent of one another, but he has regarded these effects as probably being the rearrangements themselves, produced according to the contact hypothesis. For he has considered it unlikely that the effects should persist individually in the sperm for so long a time and nevertheless be able to interact with one another later, as required on the view of independent breaks. Thus the contact view would seem the more plausible one, if the facts regarding dosage, to be discussed again in § III, were not taken into consideration.

(d) *Lack of effect of intermissions in treatment*

In extension of the results above reported, evidence may next be presented which shows that the individual ion-effects in question must persist independently of one another within the spermatozoa even during the periods when the latter are not being treated, no matter how long these periods may be. For when, in the "timing experiments" with X-rays, done with Dr Makhijani, a given dose of radiation (1500 r.), applied to impregnated females, was fractionated into four quarter-treatments of $1\frac{1}{2}$ min., separated from one another by intervals of a week, spent at 8° C., it gave sensibly the same translocation frequency in the spermatozoa as when the same total dose was administered in the form of one continuous treatment of 6 min., followed by immediate breeding of the flies. These results are shown in Table IV (first two columns of data).

TABLE IV

Timing experiment

(X-rays, 1500 r., applied to impregnated females)

	Continuous treatment, immediate breeding	Treatment in four fractions; 1 week at 8° C. between each, and following last fraction	Continuous treatment; breeding delayed 30 days at 8° C.
No. of cultures	770	675	778
No. of II-III translocations in sperm	19	18	19
% of translocations	2.5 ± 0.6	2.7 ± 0.6	2.5 ± 0.6

Finally, in a third series of the same "timing experiment", also recorded in Table IV (last column on right), the flies (impregnated females,

14 *Structural Change in Chromosomes of Drosophila*

as before) which had been given the same total dose, in the form of the 6 min. continuous treatment, were kept for 1 month (at 8° C.) before being bred. Again there was sensibly the same frequency of translocations in the spermatozoa. There is, then, no gradual disappearance of the individual ion-effects in the sperm, nor any interaction or combination occurring between them during this long period between treatment and fertilization. In all this time, the effects of the individual "fortunate" ionizations must somehow persist, independently of one another.

All this parallels facts already well known for gene mutations, which were in fact followed at the same time in our experiments, as a check on the dosage. And so, if we had no further data on the frequency of induced structural changes, we might conclude that, like the gene mutations induced by radiation, each sectional rearrangement must be the product of an individual "fortunate" ionization. For at any rate the facts do prove that the observed structural changes are somehow derived from individual ion-effects which persist separately and independently throughout the duration of life of the sperm, i.e. until fertilization at least.

III. THE $3/2$ POWER RULE: COMBINATION OF THE EFFECTS OF INDIVIDUAL IONIZATIONS AFTER FERTILIZATION

(a) *The frequency-dosage relation for ordinary translocations*

And yet the above ion-effects in the sperm cannot be the structural changes themselves, in their final form: they must be antecedent effects of some kind, which do not stand in one-to-one correspondence with the structural changes. For despite the above results the frequency of translocations and other gross rearrangements produced by irradiating spermatozoa has been found in the present work, as in the earlier work of ourselves and collaborators (*op. cit.*), not to be proportional to the total number of ionizations, i.e. to the "dose", but to nearly their $3/2$ power, when the total dose is varied from 1000 to 4000 r. The data of Sidky and the author, given in Table V, show this clearly for translocations of the major autosomes.

(b) *The relation for gross structural changes involving limited regions*

Moreover, the data of Makki and the author, given in Tables X, XI (p. 38) show the same thing for translocations of the fourth chromosome affecting the cubitus interruptus character, for gross deletions of the X-chromosome, and for rearrangements giving a mottled expression of

the white locus, i.e. for the very types of gross structural changes for which a linear frequency-dosage relation had been most definitely claimed by adherents of the contact hypothesis (Demidova, 1937; Heptner & Demidova, 1936; Khvostova & Gavrilova, 1935, 1938). It is important to note that these frequency-dosage relations were observed by us in the same collection of flies in which at the same time the other mutations observed—gene mutations and minute rearrangements, allelic to given “visible” mutations—were found to show a simple linear frequency-dosage relation. The latter observations, then, served as a check on the reliability of the experiment for revealing a proportional relationship where it was really present.

TABLE V
*Frequency-dosage relation for translocations of type II-III
for range between 1000 and 4000 r. (X-rays)*
(Adult males irradiated)

Dose in r.*	No. of cultures	No. of trans- locations	% of trans- locations
4000	1,373	114	8.30 ± 0.76
1000	10,196	118	1.16 ± 0.12

Expected percentage of translocations for 1000 r., calculated from percentage for 4000 r., on basis of different exponents (x), where percentage varies as (dosage)^x

x	1	1.5	2.0
% translocations	2.07 ± 0.19	1.04 ± 0.10	0.52 ± 0.05

Observed % = 1.16 ± 0.12 , representing $x = 1.42$.

* The absolute dosage determination is subject to considerable error here, but the treatments were so given that, in any event, the high dose was very nearly four times the low dose.

There are a number of possible explanations for the aberrant results reported by the workers previously mentioned for the cubitus interruptus and other classes of gross structural changes. One source of error may have lain in differences in the average temperature between their high- and low-dose series during the time in which the flies were developing, since temperature markedly affects the “penetrance” as well as the relative viability of some of the characters concerned (notably, that of “cubitus”). In our experiments the two contrasted lots in a given experiment were always raised at the same temperature (a cool temperature, for cubitus), and the numbers in the two lots bore approximately the same ratio to one another in the different experiments that were summated. Another source of error lies in the fact that the measurement and control of absolute dosage presents considerable difficulties, so that special pre-

cautions are needed to ensure the two doses having just the right values relatively to one another. This we secured by Offermann's technique of dividing our low-dose group into four equal parts, each treated simultaneously with a different quarter of the high-dose treatment. And here again it is important that the ratio of high- to low-dose offspring examined is approximately the same for the different experiments which are summarized. Finally, it should be noted that in some of the reported work the data do not well bear out the relations claimed. For example, an analysis of the data of Heptner & Demidova (1936) shows (contrary to their claim) a rise in the frequency of gross deletions, from 4000 to 6000 r., significantly higher than expected on a linear relationship, whereas the apparent gene mutations studied at the same time do not show this phenomenon; again, the numbers of "mottleds" found by them were too small to be of much significance.

Bauer *et al.* (1938), accepting the reported proportionality relationship for cases like "cubitus", argue that this is only what might be expected, even on the breakage theory, for structural changes of such a nature that one of the breaks is required to occur in a region of very limited extent (here, the fourth chromosome), while the other is allowed much latitude. For in such cases, they say, there might always be enough breaks of the second kind available, and so the frequency of the combination might directly depend on the frequency of the breakages in the limited region alone, and hence might, like the latter, be proportional to the dose. It will be seen that this argument presupposes that the frequency of breakage in the less limited regions is, at both the doses used, at such a high level that no further rises would have a detectable effect. In fact, however, such a level would not be reached until the great majority of germ cells contained potentially effective breakages, and in that case there would certainly be more than 10 % of detectable translocations between the major chromosomes. Thus the consideration proposed would not be applicable to the experiments in question. Considering the matter from another point of view, it is to be expected that, at all doses, the ratios of different kinds of two-break rearrangements to one another should be the same, no matter what chromosome regions they involved. Hence the results we have observed for cubitus and the other special cases are in fact those to be expected on the breakage theory. We are, however, running somewhat ahead of our argument in taking up this question here, and we may now return to a consideration of our $3/2$ power results in general, accepting them as applicable to all the kinds of gross structural changes in question.

(c) *The dissociation of the process of structural change
into two distinct stages*

Now if, in general, individual "fortunate" ionizations produced the gross structural changes independently of their fellow ionizations, the above $3/2$ power results with regard to gross structural changes would have been impossible. For in that case the number of the structural changes would be equal to the number of ionizations, multiplied by the chance that any given ionization would be "fortunate" (i.e. effective in producing a change of the type in question), and so the frequency of the structural changes would be simply proportional to the frequency of the ionizations, that is, to the dose, just as the frequency of gene mutations is. An exponential rule (more accurately, one with an exponent above 1) proves, on the contrary, the occurrence of some kind of interaction between the individual ions, or between their effects, even though we have just proved that no such interaction can occur within the spermatozoon.

It necessarily follows that the deduced interaction must occur at a later stage than the spermatozoon, that is, after fertilization. It must, however, usually occur before the first cleavage division, otherwise individuals mosaic for the structural changes would be commoner than whole-mutants. Thus, when taken in connexion with one another, our two seemingly antithetical findings—(1) the independence of the ion effects in the spermatozoa, as shown by the lack of influence of their time and space distributions, and (2) their eventual interaction, as shown by the $3/2$ power rule—effect a dissociation of the process of origination of structural changes into two distinct stages, having a considerable time interval between them, sometimes more than a month. In the first stage, occurring in the spermatozoon, there are antecedent changes, each of which is dependent on an individual fortunate ionization, just as a gene mutation is. Each of these effects is then retained as it is, not undergoing further change, until fertilization. Soon after that, in the second stage, some at least of these antecedent effects undergo combination with one another, in such a way that the $3/2$ power relationship results.

(d) *The sufficiency of the breakage-first mechanism
in the genesis of the $3/2$ power rule*

Here, however, the question arises, why does the frequency of structural changes follow the $3/2$ power of the frequency of the ions, instead of the square? For the latter is the relation to be expected, as an approximation, for all ordinary "double accidents", i.e. for the coincidence of

two independent and equally likely events, each of which is rare. (The greater the rarity, that is, the lower the frequency in question, the more nearly negligible becomes the chance for the coincidence of more than two of the events, and so the more accurate becomes this approximation, as noted on p. 19.) Can it be that gross structural changes fail to obey this square rule because they are a mixed lot, some representing combinations and others individual ion-effects? Or are they all to be regarded as combination effects, which seem to depart from the usual square rule for "double accidents" only because (on account of their relatively high frequency) many of the "coincidences"—an increasing proportion with increasing dose—are lost to view in the occurrence of "multiple coincidences", as well as through the inviability of the products formed on these occasions, as mentioned in the introductory section? It is evident that calculations are necessary here, to determine whether the departure from the square rule to be expected on these grounds is of such magnitude as to give rise to the relation actually observed.

If we assume that the individual ion-effects occurring in the sperm, which by their combination give rise to the observed structural changes, are breakages, or potential breakages, and that their combinations occurring after fertilization are the fusions of the broken ends, then the calculations in question are largely worked out for us already, and their results presented in the valuable tables of Catcheside (1938 *a*). On consulting these tables, and then computing the relations between the "expected values" there shown, we find that, for a range of frequencies of (viable) translocations similar to that observed in our experiments, these "expected" frequencies do in fact increase very nearly as the $3/2$ power of the individual breakage frequencies (that is, of the dose, where individual breaks result from individual ionizations). The relation falls short of the square, first, because of the so-called "saturation effect"—cases of multiple rearrangements being scored like double combinations—and secondly, because in these cases of multiple combinations there is a disproportionately increased chance for inviable products (acentric and di- or polycentric chromosomes) to be formed.

It is true that certain premises have been assumed for convenience in making these tables, which there is reason to believe do not hold. One of these is the assumption that fusions are random, whereas, as we shall see presently, there is reason to believe that an original proximity of the broken ends probably favours their union. Another is the admittedly invalid restriction, assumed for convenience by Catcheside, that there cannot be more than one break in each chromosome. However, in col-

laboration with Pontecorvo, I have tested the effect of removing these restrictions and find that, over the range of translocation frequencies dealt with by us (i.e. for doses of 1000–4000 r. in our material), the relations calculated by Catcheside remain true for translocations to a sufficiently near approximation for our present purpose. In a word, it turns out that these expected frequencies of structural changes vary nearly as the $3/2$ power of the individual breakages, that is, of the dose. This holds, moreover, for inversions and deletions as well as translocations. Hence the observed $3/2$ power relation does not really give ground for the suspicion (which I had once entertained (1936 b, 1938)) that some of the gross structural changes might have been occasioned by single ionizations that somehow gave rise to both breaks at once.

(e) A further test—the rise in the exponent at lower doses

There is another way of testing whether the gross structural changes are of mixed origin, some from combinations and others from single ion-effects. That is to find the frequency-dosage relation for a lower range of doses than those hitherto used. At lower and lower doses, the frequency of cases of multiple-break rearrangements expected as accidental combinations of independent breaks decreases rapidly, in comparison with the ordinary double-break rearrangements. Virtually all rearrangements then become those of the simplest possible type, namely, double combinations. With the source both of “saturation effect” and of differential viability thus removed, the frequency of viable structural changes must rapidly approach proportionality to the power 2—that is, to the square—of the dosage, as calculations which I have made in collaboration with Pontecorvo, extending the tables of Catcheside in the direction of lower dosage, clearly show. This, then, is the relation to be expected at low doses if *all* gross structural changes arise by combination, occurring subsequently to breakage, between broken ends derived from independently produced breaks.

On the other hand, if any appreciable part of the gross structural changes were single-ion effects, this part, being linearly proportional to the dose, would, as the dose decreases, decrease much less in frequency than the rest (since the latter as above shown diminish as the square of the dose). Thus the single-ion rearrangements would become an increasingly prominent fraction of the whole as the dosage was reduced, and, becoming preponderant in number at lower doses, would force the frequency-dosage curve as a whole to approach their own graph—a straight line—at such doses. That is, the exponent in question, instead of

20 *Structural Change in Chromosomes of Drosophila*

rising from $3/2$ towards 2, at lower and lower doses, as it would if all structural changes were combination-effects, would fall from $3/2$ towards 1 (Muller, 1938).

It is hard to get significant data for the very low frequencies given by the lower doses, but, as Table VI shows, there is certainly no drop of the exponent towards 1 in the range of lower doses from 1500 down to 375 r., as expected on the view that some of the breaks involved in gross structural changes are interdependent. On the contrary, the smallness of the numbers of translocations obtained in itself makes it probable that the exponent is nearer 2 here than at higher doses, as expected on the unmodified conception of all gross structural changes being combinations of independent breaks. For while the value obtained in the series at

TABLE VI
*Frequency-dosage relation for translocations of type II-III
for range between 375 and 1500 r.*

Rays	Dose in r.	No. of cultures	No. of trans- locations	% of trans- locations
X	375	1563	5	0.32 ± 0.14
γ	400	1803	2	0.11 ± 0.08
Sum of above		3366	7	0.21 ± 0.08
X	1500	2223	56	2.52 ± 0.34

Expected percentage of translocations for 375 r., calculated from percentage for 1500 r., on basis of different exponents x , where percentage varies as (dosage)^x

x	1	1.5	2
% translocations	0.63 ± 0.08	0.31 ± 0.04	0.16 ± 0.02
Observed % = 0.21 ± 0.08 , representing $x = 1.8$.			

375 r., as compared with 1500 r., does happen to agree with the expectation for the $3/2$ power rule, it does not disagree to a statistically significant extent with the value to be expected on the square rule, while at the same time the value obtained in the series at 400 r., which agrees with the expectation for the square rule, does disagree significantly with the expectation based on the $3/2$ power rule. This indicates that the agreement with $3/2$ in the former series was accidental. Most probably, then, the exponent at this range of doses has a value intermediate between $3/2$ and 2, i.e. about 1.8, which is the value indicated when the two series of results are combined. And at any rate we can say that these experiments give evidence that the exponent rises nearer to 2 at lower doses, as expected for changes all of which arose from combinations of independently produced breaks.

IV. OTHER EVIDENCE THAT BREAKAGE PRECEDES UNION

Although the contrast between our results on the independence of the ion-effects in the spermatozoa (shown by the intensity-time, etc., data) and on their interaction at some other stage (shown by the total dose data) demonstrates clearly that the structural changes occur in two steps, this alone does not prove that the single-ion effects are the breakages and the combination-effects the unions of broken ends, far-fetched though any other interpretation of these facts would seem. However, this particular interpretation was taken as the basis for the calculations whereby the $3/2$ power relation was arrived at as the expectation for ordinary doses, as well as for those calculations which gave the square relation for lower doses; hence these more specific agreements of observation with theory may be taken as indicating the correctness of the identification of the single-ion, "primary" effects with the breakages and of their interactions with the fusions. But at the same time, it would be desirable to obtain evidence for this premise from other angles.

(a) The frequency-dosage relation for losses of chromosomes

One direction in which we have sought such evidence lies in studies designed to investigate the frequency of losses of chromosomes in relation to total dosage. If the primary effects were really breakages, there might be some fragments, formed by simple breakage, which failed to undergo union in due time, and many of these cases should eventually entail losses of the whole of the affected chromosome, including both its fragments. For, being without telomeres at one end, the twin chromatids derived from each such fragment by its division may be expected, where they do not undergo other unions, eventually to unite with one another at their broken ends to form dicentric and acentric chromosomes, in the way described by McClintock in maize.¹ Now the frequency of these losses, referable to individual primary breakages ("simple breaks") which failed later to undergo union, should vary roughly linearly with the dose, if we can as a first approximation neglect the influence, on the opportunity for the occurrence of such cases, caused by some of the broken ends combining with ends derived from other breaks. We can probably do this, since union of opposite broken ends derived from the same break (restitution) probably occurs far oftener, at breakage-frequencies as low as those dealt with, than union between broken ends

¹ According to her recent account, however, the ends can become "healed" in sporophytic tissues, but that this cannot occur in *Drosophila* is indicated by the genetic evidence against the persistence of "simple breaks" in this form.

derived from different breaks, and the tendency to undergo these restitutions, being in itself constant, could not interfere with a linear frequency-dosage relation for the cases of failure to unite. If, on the other hand, the influence of other broken ends were not negligible in this connexion, its effect would be to lessen the opportunity for the losses (due to failures of union) at higher doses more than at lower doses, and so it would reduce the frequency-dosage exponent for losses even below 1, making it still more different from the relation found for other gross chromosome changes. In any case then, on our view, the losses should give a much lower exponent than the surviving structural changes.

Unfortunately for a test of this question, losses of either of the large autosomes are lethal in their effect, and even losses of the fourth chromosome result in offspring ("haplo-fourths") of very low viability, while losses of either the X- or Y-chromosome from the spermatozoa give rise to flies that differ from the class expected to arise from the Y-bearing sperm only in their non-possession of a Y-chromosome. As the presence or absence of a Y-chromosome ordinarily produces no visible difference in the phenotype, a special genetic technique had to be set up in our experiments, for the purpose of obtaining such a difference. This involved the use of a dominant, mosaically expressed allele of brown eye (bw^{F^4}) in one of the second chromosomes, as detector of the Y-chromosome, and the presence of vermilion (v) in attached X-chromosomes, to intensify the effect of the brown allele. Flies homozygous or hemizygous for v and heterozygous for bw^{F^4} have eyes that are almost typically vermilion if an extra Y-chromosome is present (as in XXY females and XYY males), but their eyes are much lighter and more distinctly mosaic if no extra Y is present (as in XX females and XY males). In this cross, shown in Fig. 1, the fathers were X-rayed, and the frequency of loss of their X or Y was determined by counts of the number of light-eyed flies among the female offspring not showing Curly wings, and hence carrying bw^{F^4} . In many cases the absence of the Y was further verified by tests of the fertility of sons of such females, since males without a Y are sterile.

The preliminary data, obtained by Singh, Alexander and the author last spring (1939) indicate that these chromosome losses increase in frequency no faster than the dosage. This result, so unlike that for two-break or multiple-break rearrangements, thus tends to confirm the "breakage-first" hypothesis from another angle. The result, moreover, shows that only a small proportion of broken ends thus fail to rejoin in due time, since at these doses there are many more translocations of a given chromosome than losses. This in turn justifies the premise used in

the calculations of expected frequencies of structural change, that practically all broken ends undergo union. This type of investigation is now being extended by Pontecorvo and the author, with variations designed

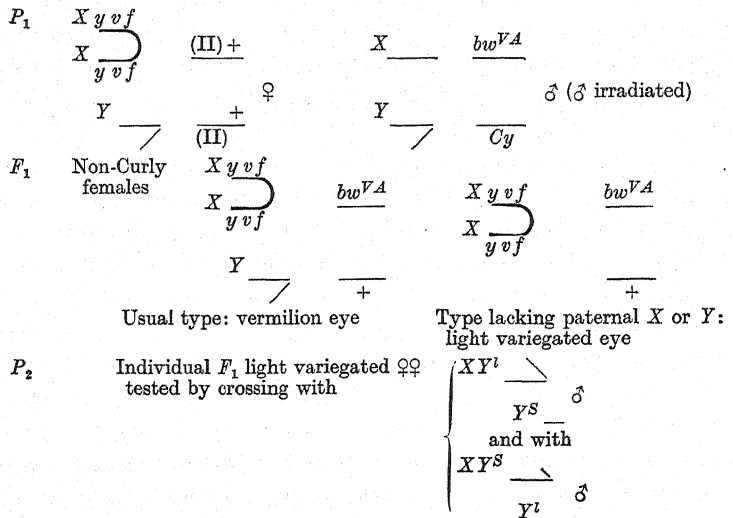


Fig. 1. Detection of loss of paternal X or Y. F₂ and P₃: tests of fertility of sons from both above P₂ matings, to determine whether Y^S or Y^l or neither was present in light variegated F₁ ♀.

TABLE VII

Frequency of whole-chromosome losses in relation to dosage

Dose	No. of Cy ⁺ ♀♀ (F ₁)	Exceptions lacking paternal X and Y		% induced loss
		No.	%	
ca. 2000 r.	1994	32	1.6 ± 0.3	1.4 ± 0.3
ca. 500 r. (¼ of above)	5978	30	0.5 ± 0.1	0.3 ± 0.1
Controls	2461	5	0.2 ± 0.1	—

Percentage of induced exceptions expected for 500 r., calculated from percentage observed for 2000 r., on basis of different values of exponent [x], where percentage varies as (dosage)^x.

x	1	1.5	2.0
%	0.35 ± 0.1	0.17 ± 0.04	0.09 ± 0.02

to give evidence from still another direction that these total losses of given chromosomes are really caused by their simple breakage.

In the abstract of the paper which Bauer (1939) was to have read at the Seventh Congress, mention is made of results which may be of an essentially similar kind. Here the frequency of loss and/or dominant

lethality of the X-chromosome derived from irradiated *Drosophila* sperm was estimated by the amount of change caused in the sex ratio. A linear relation to dosage was found, like that in our work reported above. Bauer, too, regards the losses as due to individual breaks, and so, taking their apparently linear relation to dose into consideration, along with an exponential relation for completed rearrangements (though the exponent appears to be too high, see p. 7), he is led to accept the breakage-first theory.

It should be mentioned that in this work Bauer used a ring-shaped X-chromosome, in which of course exchanges between chromatids (union of the left fragment of one chromatid with the right fragment of the other and vice versa, as in crossing-over) must result in dicentric chromosomes that are lost. Hence he appears to attribute the losses of these X's to exchanges of this kind that follow breakage of the chromosome. While this mechanism is no doubt in part responsible for the losses of such chromosomes, there must also be losses caused by union of adjacent broken ends of the *like* fragments of sister chromatids, just as occurred in our non-rings discussed above. Both categories of loss can legitimately be added together, however, for the purpose of determining the frequency of the individual chromosome breaks, since the breaks are followed by both types of effect.

Unfortunately, however, Bauer's sex-ratio technique fails to distinguish between the reduction of the ratio of females to males caused by these X-chromosome losses (of all kinds taken together), and that caused by the induction in the X of dominant zygote-lethal changes of various possible types (gene mutations, and gross and minute structural changes). Until a distinction into these two major components can be made, it would seem premature, on the basis of this evidence, to speak of the losses by themselves as having a linear frequency-dosage relation. In fact, as the losses might have caused but a minor part of the observed effect, the linearity of the curve as a whole might merely have been an expression of the already known linearity of the curve for gene mutations and minute rearrangements.

There is reason to infer that the cytological data of Marshak (1935, 1936, 1937), on the frequency of "abnormal mitoses" containing bridges and fragments in the first division following irradiation in root tips and anthers of various plants, as well as in animal tissues, really deal with the phenomenon of loss of chromosomes caused by simple breakages in the way above depicted: (1) chromosome breakage, (2) chromosome reduplication to form sister chromatids, (3) union of adjacent broken ends

of like fragments of sister chromatids, (4) bridge formation by the resultant dicentric chromosomes at the succeeding mitosis, and (5) final loss of the dicentric and acentric parts. This interpretation brings Marshak's finding that the frequency of these "abnormal mitoses" is linearly proportional to the dosage (cited on p. 6) into complete agreement with our own findings and more especially with those reported in the present section, although Marshak's own interpretation is that two sister chromatids (formed by a division preceding irradiation) were both affected by the passage of a single electron, in such a way as to allow them to come into contact, their exchange of connexions and breakage being secondary to this.

In our view, contrary to Marshak's (which we further criticize on pp. 37 and 45-6), his finding of a sensitive period for the induction of these bridges, in early prophase about 3 hr. before the metaphase of mitosis (Marshak, 1937), constitutes evidence that the chromosomes are not in the reduplicated ("divided") condition at all until just after that time. Having then divided, union between adjacent broken ends, followed by bridge formation, should be far more frequent than any other type of structural change (since it requires but one break, and involves union of nearby parts), and so it should dominate the frequency-dosage curve of the "abnormal mitoses" that follow, and cause it to be linear, like the curve of the breaks themselves. Breaks produced earlier would tend to undergo restitution before chromosome doubling, and breaks produced after doubling should usually at least involve but one chromatid; hence in neither of these cases would nearly so many bridges or fragments be formed as at the above critical time.

(b) Chromosome mosaics and biparental rearrangements as evidences that fusion of broken ends may be delayed

If the above interpretation of the total losses of given chromosomes is correct, broken ends sometimes fail to fuse before chromosome division. When that happens, however, it is to be expected that the sister fragments of the chromatids formed by this division will not always unite with one another, thus resulting in these total losses of a chromosome, but that sometimes the fragments of one chromatid will unite in a different way from those of the other chromatid (i.e. that they will unite with different broken ends), and that at other times one fragment will fail to unite while its sister fragment does undergo union, either with a broken end derived from the same or with one from a different breakage. This explains, as variant results of one more general phenomenon, the

formation of the chromosome mosaics of various kinds reported by Patterson (1931, 1932, 1933, 1935, 1938) from irradiated sperm, including both the gynandromorphs in which the entire irradiated X is absent from one-half of the fly and the individuals mosaic for deleted and other structurally changed chromosomes derived from the treated sperm.

The above is also the only plausible explanation for some cases of duplication, e.g. "turn backs" like dominant eyeless or the "bull" near the left end of the X-chromosome (see discussion in paper of Offermann, 1936; see also Bridges, 1935 *b*, and Kaufmann & Bate, 1938) in which two identical regions, known or inferred to have been derived from sister chromatids, have become attached together. These cases likewise must have originated as mosaics. In them (unless we conceive the stresses between sister strands here to have been similar to those between homologous strands at crossing-over, which is very unlikely) the extreme exactitude of the correspondence between the points of breakage and junction of the two pieces constituting the duplication furnishes a virtual proof that these breakages occurred as a single breakage, before chromosome division, while the fusion, being between chromatids, must of course have occurred after that division. Thus these cases in themselves provide a strong argument for the breakage-first conception.

It should be noted that the above interpretation makes unnecessary the assumption adopted by Patterson, for the purpose of explaining why some structural changes from treated spermatozoa appear as mosaics and others not, that the chromosomes are divided in some sperm but not in others. If we did not take into account mosaically arising ("fractional") "gene mutations" the chromosomes could best be assumed to be undivided in all the sperm, as it seems more reasonable to infer on other grounds as well.¹ In that case there would also be no convincing evidence

¹ It is a very interesting question why fractional mutants occur among the gene mutations as well as among the chromosome mutations produced by irradiation of spermatozoa, and seemingly in similar proportions. Does this mean (1) that the chromosomes are divided in some sperm but not in others, or (2) that, the chromosomes being divided in all sperm, most breaks and gene mutations are produced in both chromatids at once by an identical double action of the effective ionization, or (3) that, the chromosomes being undivided in all sperm, the gene mutational changes, like the rearrangements of sections, are not fully consummated until after fertilization and chromosome division, the effects meanwhile persisting in some intermediate stage, subject to restitution? If, as we believe, the former two hypotheses are less likely, we are confronted with one more suggestive similarity between the mechanism of production of "gene mutations" and of known structural changes, that again raises the question whether they are not size variants of essentially the same or closely related processes. In this connexion Stadler & Sprague's (1936) finding that fractional mutants are produced in higher frequency, relative to whole mutants, by ultra-violet than by X-rays, affords a possible further means of approach.

as yet, in our results, for the inference that a single ionization may break two sister chromatids at once. And examination of the published cytological evidence relating to other organisms does not convince us that such double breakage has yet been proved in these forms either; in fact, division following breakage seems better to explain many at least of the reported cases. It seems, to be sure, a not unreasonable inference, from the fact (§ V) that one ionization can break two points near together in the same chromonema, that it could probably break two nearby points in sister chromonemas as well, especially during the favourable stage, just after chromosome doubling, when the sister strands still lie very closely apposed. Yet, without empirical evidence, we cannot yet be sure that the effects of an ionization would be transmitted as well between two chromonemas as along them (or as well between them as across the space separating one rung of a given helix from the next rung).

Striking evidence that the broken ends, formed in the sperm, can wait for union not only till after fertilization and after chromosome division but even until after mixing of sperm and egg chromosomes has occurred, is supplied by the finding of a translocation between a paternal chromosome (the third) from an irradiated sperm and a maternal one (the Y) which evidently broke spontaneously in the non-treated egg. This case, of a type for which we had long been searching, is reported by Sidky (1939) in a parallel paper at the Seventh International Genetics Congress.

(c) *The frequency relations of multiple exchanges*

Another line of attack on the mechanism of structural changes is through a study of their anatomy. On the view of prior contact or close approximation of the parts entering into an exchange of connexions we should ordinarily expect only an exchange of two threads or parts of a thread at any one point, that is, if $1L$, the left broken end derived from break 1, united with $2R$, the right broken end from break 2, then $1R$, the right broken end from break 1, would have to unite with $2L$, the left one from break 2, not with a broken end derived from a third break. Nevertheless triple and still more multiple recombinations having a cyclic type of mutual exchange (e.g. $1L-2R$, $2L-3R$, $3L-4R$, $4L-1R$) are not infrequently found. On the contact or approximation view these would, as I have pointed out previously (1932), require the seemingly strange coincidence of the meeting and breakage of the third and other threads at exactly the point where the first ones met and recombined. Offermann has suggested that the contact view might be saved here by the special assumption that other threads tend to get caught in a place where two

threads are already crossed or in contact, and Dubinin and his co-workers (Dubinin & Khvostova, 1935; Kirssanow, 1937) accept this idea of a "knot", as they call it, for their cases, some of which are extremely compound.

In this connexion it is to be noted that in the spermatozoa—which provide the main material for induction of structural change hitherto studied with reference to this point—the chromosomes are so crowded together that such "knots" might be almost ubiquitous. That is, a large proportion of the ions capable of causing structural change at all might, on this view, be in such a position as to be able to affect several chromosomes (or chromosome parts) at once. However, the greater the number of threads thus conceived of as simultaneously affected and exchanged, the further and in the larger number of directions must the effects of the individual ionization be supposed to extend. On this view, moreover, no larger proportion of these multiple exchanges would be produced in this manner at higher doses than at lower doses, in proportion to the ordinary double exchanges. It is true that, even so, there would, with increasing dose, be a certain increment of complex cases of another kind—i.e. those which had originated by the coincidence of two or more separate ionizations. But this increment should be relatively small, compared with that expected if all the breaks of gross rearrangements arose from separate ionizations, and the amount of it should be calculable, at least approximately, from the frequency of exchanges in general. In view of these considerations, a study was undertaken by Sidky and the author to determine to what extent the ratio of identifiable multiple to double (or total) exchanges varied as the dosage was varied.

The fact was utilized that all ordinary (double-break) translocations of a ring chromosome with another chromosome result in a union of the two chromosomes to form a single dicentric chromosome, which is unable to survive. To be viable, a translocation of a ring chromosome must involve at least three breaks: either two breaks in the ring, with deletion of the acentric piece, which is inserted into the other chromosome at the latter's point of breakage, or vice versa, two breaks in one arm of the other chromosome, with insertion of its deleted piece into the ring. A mutual deletion-insertion, from each chromosome into the other, which would involve four breaks, would also allow the product to live; this could, however, arise by two separate double exchanges as well as by true multiple exchange.

For the purpose of the experiment the ring *X*-chromosome found by Beadle, designated as Xc^2 , was used. Males containing this chromosome

were crossed, after irradiation, to females with attached X's and with recessive markers (*bw* and *e*) in the major autosomes, and the F_1 males were back-crossed to females like the mother. The summarized results from two different doses are shown in Table VIII. Here the numbers of translocations involving the ring chromosome with either of the major autosomes are shown, and also the numbers involving the major autosomes with one another. The latter may be taken as a fairly reliable measure of the frequency of translocations in general, including both multiples of all types and ordinary double translocations, although the doubles predominate at the doses used. Any change in the ratio of multiple to double translocations associated with change of dosage should

TABLE VIII
Translocation frequencies in relation to radiation dose

Heavy dose (H) = 4 × light dose (L).

Series	Dose	Type of X-chromo- some*	Fertile F_1 cultures	Translocations involving			
				X-2	X-3	X-2-3	2-3
1	L	R	1132	0	0	0	12
	H	R	160	0	1	1	14
	H	+	26	0	0	0	3
2	L	R	1176	0	0	1	28
	H	R	156	1	0	0	19
	H	+	59	1	0	0	5
3	L	R	863	0	1	0	4
	H	R	141	0	1	0	6
	H	+	54	0	2	0	2
4	L	R	1238	0	0	0	8
	H	R	136	0	0	0	8
	H	+	83	0	0	1	7
5	L	R	1492	0	0	0	20
	H	R	195	0	0	1	20
	H	+	99	2	1	0	10
Total	L	R	5901	0	1	1	72
	H	R	788	1	2	2	67
	H	+	322	3	3	1	27

* R=ring (Xc^2); +=non-ring.

accordingly be even more pronounced than any change found in a comparison of our ring chromosome translocation frequencies with our II-III translocation frequencies.

It may be seen that there is a difference between the ratios of ring to II-III translocation frequencies at the two doses in the direction expected on the breakage-first conception. While the data are not yet large enough to make the difference secure, from a statistical standpoint, nevertheless the increase of the ratio of ring to II-III translocations with dosage is more pronounced than would be expected merely as a result of the accidental concurrence of two essentially separate double translocations.

Cases of the latter type, involving the ring in a combination of two translocations with any one of the autosomes, should, according to the Poisson rule, have a frequency approximately half of the square of the frequency of ordinary (double-break) translocations of a non-ring *X*-chromosome with that autosome. From the present and previous work with non-ring *X*'s, which shows that the frequency of translocations between *X* and II detectable by this method (mainly double-break exchanges), as well as the frequency of those between *X* and III, is not more than one-sixth the frequency of those between II and III, we may calculate that all cases detectable in this way which have a combination of two double-break translocations between a ring and the same major autosome should not exceed 0.0002 % at the lower dose used and 0.01 % at the higher dose. Considering the number of cultures, these percentages would here be represented only by *ca.* 0.01 individual at the lower dose and 0.1 at the higher. Hence the observed increment of ring-chromosome translocations with rise of dosage could not possibly be referred to this cause. Now, the fact that a large part of the ring chromosome translocations found even at the higher dose are cases of actual triple exchange rather than of two double exchanges is further indicated by the observation that in several of them the aneuploid of one or the other of the two complementary types is able to survive, whereas it is highly unlikely, in cases of two double exchanges, that both transferred sections would be small enough to permit this. Some of the translocations are now being studied cytologically to confirm these conclusions.

All in all, then, this experiment, so far as it goes, provides evidence that the multiple-exchange translocations themselves, and not merely the combinations of double-break translocations, increase rapidly in frequency with increase of dose. And this in turn would lead to the conclusion that the different breaks involved in the multiple-exchange translocations have usually originated independently of one another, that is, as a result of separate ionizations, rather than through the mechanism of one ionization that affected the several chromosomes simultaneously. Further work of the same kind is now under way, to provide more decisive data on the question.

The results of this experiment have also been examined to determine to what extent the frequency of the multiple-break cases involving the ring chromosome agrees with an expectation based on the complete randomness both of breakages and of unions of broken ends. The mathematical analysis is too intricate to be presented here, but it may be stated that the results indicate the occurrence of a larger number of ring-

chromosome translocations than expected on the basis of complete randomness.¹ The results show that only a small part of this excess can be due to the inclusion of cases of minute rearrangements that happen to be combined with gross rearrangements (in these the two breaks that are close together are, as will be shown in the next section, dependent on a common cause and thus depart from randomness). The excess of other multiple-break cases must be due to some kind of influence of proximity of the broken ends (in the positions they had at the time of breakage) on the chances of their ultimate union with one another. This might work out in several ways to increase the number of multiple (ring chromosome) translocations. One way would be by the favouring of the union of broken ends belonging to the same rather than different chromosomes and chromosome arms. Another way would be by the restitution of original chromosomes (reunion of broken ends derived from the same break) occurring more readily than new combinations.

*(d) The overlapping of the two stages of structural
change in ordinary cells*

Although the fact that the broken ends do not rejoin during the spermatozoon stage has afforded a favourable condition for the genetic analysis of the process of structural change into the two components, breakage and union, the inhibition of fusion encountered in the sperm must not be supposed to represent a typical condition. For the condition of the chromosomes in spermatozoa is not the same as in ordinary cells, except perhaps in those which are in the middle of mitosis. But if we apply our conception of breakage before union, gained from the above experiments with *Drosophila* spermatozoa, to ordinary cells, then the fact, long observed by experimental cytologists, that irradiation during ordinary resting stages is followed by the appearance of structurally changed (as well as fragmented) chromosomes at the ensuing mitosis must mean that chromosomes broken during the resting stage can undergo union of their broken ends during the same period, to form new combinations. It is in agreement with this that chromosomes broken in the spermatozoon stage can unite within the paternal pronucleus of the fertilized egg, for this also resembles a resting stage. Moreover, in *Drosophila* itself

¹ Bauer *et al.* (1938) also report an excess of multiple-break cases involving more than one break in the same chromosome, as compared with the expectation calculated from double breaks on the basis of random breakage and union, but when allowance is made for the higher chance of viability of multiple break cases in which the breaks are concentrated within the same chromosome, as opposed to those in which they are scattered in different chromosomes, the excess found by them no longer appears significant.

it has been known for some time that structural changes may be produced by irradiation of ordinary cells, such as spermatogonia, oögonia, and embryonic somatic cells. Here the union of pieces must occur at some other stage than that of fertilization and, by analogy with the above-mentioned cytologically observed cases, this is probably the resting stage for the most part.

If, now, irradiation is applied during the resting stage, fusions of the broken ends can take place during the time of irradiation itself, and the longer the duration of this irradiation, the more fusions will occur before it ceases. In that case, however, our principles of the independence of the effect from the timing of the treatment (Bunsen-Roscoe law and independence from fractionation) which hold in spermatozoa would fail to apply in ordinary cells with "resting" nuclei. For in the latter the union of pieces, including restitution, would proceed before all the breaks had been produced, and so some of the ends formed by earlier breakages would be deprived of the opportunity of fusing with those formed by later breakages, and restitutions and simpler combinations would thus be favoured at the expense of new and more multiple combinations, to an extent which would be the greater, the longer any given dose of radiation were protracted or (by fractionation) delayed in its completion. Such in fact are the results which Sax (1939) has recently reported in his cytological work on microspores of *Tradescantia*. Independent results of the same kind, on the same material, have still more recently been reported by Fabergé (1939). But despite the complication of the analysis here, caused by the overlapping of the two stages, breakage and fusion, direct observation of the chromosomes in the first division after treatment has provided a means whereby these investigators, in this material, have been able, independently, to arrive at conclusions closely paralleling those which we have reached in *Drosophila* through the study of the numerical consequences of the separation of the two stages in the spermatozoa and fertilized egg.

Not only the Bunsen-Roscoe law but also the frequency-dosage relation described in the preceding section is upset in material in which breakages and fusions overlap. For in such material the opportunity for the ends formed by earlier breakage to undergo fusion with those later formed will depend not merely upon the total number of breaks produced but also upon the length of time between the breakages and upon the degree of activity of the process of fusion in the meantime. Hence the longer the interval between breaks, or groups of breaks (as in fractionated treatment), in relation to the interval between breakage and fusion, the

more nearly will the frequency of structural changes become linearly proportional to the total dose, if this dose is controlled only by changing the duration and not the intensity of the treatment. But if the total dose is varied by changes in the intensity rather than in the time factor, or if, the time factor being varied, even the longest time of irradiation does not exceed the usual interval between breakage and fusion, then the frequency-dosage relation will remain an exponential one, somewhat like that obtained by irradiation of *Drosophila* spermatozoa.

In Sax's work (1938), when relatively short irradiation times were used, an exponential relation was found, and in fact the exponent turned out to be approximately the $3/2$ power. It might appear then as though this relation was an expression of just the same factors as operated in our material. Since, however, the chromosomes in *Tradescantia* were observed in the first division after treatment there were no cases lost through acentricity and dicentricity as in our material and so this factor, which was the main one in reducing our exponent below the square, was lacking. Thus the reduction of the exponent below the square, to something like the $3/2$ power, must have had some other cause in *Tradescantia*. As a matter of fact this cause lay mainly in the circumstance that, in this material, the structural changes observed were a mixed class of cases, consisting of chromosome fragments that had been derived from simple breakage, which of course followed a linear (1 power) rule, and of real rearrangements which, not yet being selected for viability, approximated a square rule (except for the "saturation effect"). In Fabergé's work too the frequency of fragments followed a linear rule. Whereas in our material simple breakage, when not followed by restitution, is always followed by loss, as previously explained, this was not true in *Tradescantia*, as there had as yet been no opportunity for loss, and it is even doubtful whether most of these cases would have undergone loss at mitosis.¹ And in addition to this major cause of reduction of the exponent there was also the factor, above mentioned, that some union of ends could take place during

¹ In view of McClintock's (1939) recent finding of the healing of broken ends in the sporophyte tissue of maize (as contrasted with gametophyte and endosperm tissue), it is questionable whether all of the chromosomes that had undergone breakage without subsequent union of broken ends would eventually be lost or not, in the *Tradescantia* material. In favour of their not all being lost, we find, on examination of Sax's tables, that, after a certain period following breakage has elapsed, the number of chromosomes which are seen to have undergone simple breakage does not seem to diminish still more with the passage of additional time, as it should if these ends had retained their capacity to fuse. That is, it seems as though the broken ends had now lost the adhesive quality which distinguished them from ends that normally were free. It would be interesting to know whether or not this apparent "healing" in *Tradescantia* is permanent.

treatment, the amount of reduction of the exponent due to this cause being dependent upon the duration of the treatment in relation to the activity of the process of fusion. The agreement of Sax's $3/2$ power rule with our own is for these reasons largely an accidental one.

It is to be observed that anything which changes the degree of activity of the process of fusion of broken ends will, in general, have an influence like that of a change of the time factor, since occurrence of more fusions in a given time will in effect be like an increase in the time in which fusions can take place. So, in Sax & Enzmann's (1939) experiments, and in the independent ones of Fabergé (1939), an increase in the temperature during or between irradiations, like an increase in the time over which the giving of a certain total quantity of radiation is spread, resulted in fewer structural changes (more restitutions).

It is to be expected, however, that a rise in temperature will accelerate some cellular processes to a different degree from others, and this may at times cause qualitative and quantitative differences in the production of structural changes. If, for example, the readiness of the chromosomes to undergo fusion with one another when their broken ends happen to come near together were increased more than the amount of gross movement of the chromosomes amongst one another, then the effect, in a given time, would be to favour restitutions and unions between broken ends that originally lay near together (e.g. derived from breaks in the same chromosome arm), to a greater extent than new combinations in general, while there would be an opposite tendency if the gross movements were increased more than the ease of fusion. Again, in the case of cells irradiated at the stage just preceding chromosome doubling ("division" into chromatids), if a rise in temperature should accelerate the doubling process more than that of fusion of broken ends, more identical sister fragments of chromatids will be formed at higher than at lower temperatures, because at higher temperatures "division" will oftener precede fusion of ends. These chromatid fragments can then undergo fusion of their sister ends (with final loss), as well as other new chromatid combinations, and so the frequency of these "chromatid changes" will increase with temperature in this case even though, in general, a rise in temperature reduces the number of structural changes, as before mentioned. This interpretation would readily explain a result of this kind which has recently been reported by Sax & Enzmann (1939) from irradiation of early prophases of *Tradescantia*, for here there was a decrease in "chromosome changes" and a partially compensatory increase in "chromatid changes" with rise in temperature.

With possibilities of the above kinds in view, an experiment was carried out by Pontecorvo and the author, independently of the last-mentioned work of Sax & Enzmann, to determine whether, in *Drosophila*, differences in temperature existing at the time of fertilization might affect the frequency of translocations obtained after irradiation of spermatozoa. In one series the temperature was varied at the time of irradiation as well, in order to enable further data to be obtained on this point at the same time. The data obtained are shown in Table IX. It will be seen that there is no evidence that temperature differences existing either at fertilization or at the time of irradiation have any effect on the frequency of structural changes in this material. But a more sensitive experiment, designed to study the possible effect of temperature at fertilization on the frequency of losses of chromosomes, caused by union of sister fragments, is now being carried out, inasmuch as relatively slight effects might be observable here which were not reflected in detectable changes in the frequency of ordinary gross rearrangements.

TABLE IX

Lack of effect of temperature on frequency of translocations (of type II-III) induced by irradiation of Drosophila spermatozoa

		Temperature at fertilization								
		13°		24°		29°		All temp. combined		
Temperature at time of irradiation	Series	No. cult.	No. trans.	No. cult.	No. trans.	No. cult.	No. trans.	No. cult.	No. trans.	% trans.
4.5 - 6.5° C.	1	245	9	180	14	—	—	425	23	5.4 ± 1.1
" "	2	274	21	872	40	858	45	2004	106	5.3 ± 0.5
" "	1 + 2	519	30	1052	54	858	45	2429	129	5.3 ± 0.5
37.5 - 35.5° C.	1	191	7	169	8	—	—	360	15	4.2 ± 1.1
Both temp. at irradiation combined	1 + 2	710	37	1221	62	858	45	2789	144	5.2 ± 0.4
		5.2 ± 0.9 %		5.1 ± 0.6 %		5.2 ± 0.8 %				
Comparison for different temperatures at fertilization										

* Comparison for different temperatures at irradiation.

V. THE PRODUCTION OF MINUTE REARRANGEMENTS BY A SPREADING OF THE EFFECT OF INDIVIDUAL IONIZATIONS

(a) *The linearity of the frequency-dosage curve*

All the above considerations apply to gross structural changes, changes in which the different breaks were far apart in the chromonema,

but it must now be recognized that minute structural changes fall into a separate category. That there is a distinct tendency for two changes at nearby points to be associated with one another was first noticed in my work reported at the Sixth Congress (Muller, 1932), and at that time interpreted as probably being due to a spreading of the effect of one ionization to both the neighbouring points in question (which were conceived as probably being more nearly adjacent, by virtue of the spiralizing of the chromonema). A systematic study of changes involving the scute locus later confirmed the inference that such cases were in fact commoner, in comparison with the rest, than chance would allow, and showed that many or all of them involve, not gene mutation as such, but chromosome breaks near together, associated with rearrangement of the small pieces (Muller *et al.* 1934, 1935). In the cases where the exchange of connexions was such as to give inversion or insertion, it became probable (by analogy with the similar phenomena in gross rearrangements, where analysis of the factors operating could more readily be carried out) that the changes in gene expression were "position effects" exerted on loci in the neighbourhood of the breaks. The long-known changes styled "deficiencies" also received their explanation in this connexion (Muller, 1935), as cases in which deletion of the minute section had resulted.

As pointed out by Bauer *et al.* (1938) and by Demerec (1939), recent studies of a series of cases of Notch deficiency by Slizynska (1938) and by Demerec (1939) give further statistical corroboration of this conception that structural changes (in this case deficiencies) below a certain size represent a class by themselves. For below the critical size they are found to be so numerous as to rise well above the curve of more or less random size distribution that is found to fit the larger deficiencies, as well as the larger rearrangements of other types.

If, now, the minute rearrangements really represent double or multiple effects of single ionizations, they should not show the exponential frequency-dosage relation that has been found for the gross rearrangements, but their frequency should, like that of gene mutations, be linearly proportional to the dose of radiation. That this is probably true might have been inferred from the linear relation shown by lethal "gene mutations", since those produced by radiation are known to include a fairly high proportion of small deficiencies (30-40 % according to data of Sacharov, 1935, and of Alikhanian, 1937, but nearly 100 % according to results of Slizynski, 1938, which are more difficult to reconcile with other findings). More direct evidence of the frequency-dosage relation

for minute rearrangements was obtained by Belgovsky working in collaboration with the author on a special class of minute rearrangements—those involving the yellow locus situated near the left heterochromatic region in the scute-8 chromosome—which have the combined advantages of comparatively high frequency and phenotypic detectability (Muller *et al.* 1937 *a*; Belgovsky & Muller, 1937; Belgovsky, 1938; Raffel, 1938). As we reported two years ago (Muller, 1937; Belgovsky & Muller, 1937) the evidence then indicated that these rearrangements follow the linear rule expected for effects of single ionizations. The summarized data of Belgovsky to date, confirming this relation, have been presented by him in a paper originally submitted for the Seventh Congress (Belgovsky, 1939). In the meantime, Makki and the author (Muller *et al.* 1939) have been obtaining further data on minute rearrangements of this type and, for comparison with them, others in the euchromatic region. The latter comprise Notches and (less certainly, since they probably include more gross rearrangements) reversions of Bar to normal eye. Our summarized data are given in Tables X and XI.

It is clear from these tables that minute rearrangements in general follow a different law from gross rearrangements. When allowance is made for the control frequency, the relation is seen to be sensibly a linear one for the results with yellow, representing the heterochromatic region. Moreover, the less ample results from the other regions agree with these as closely as would be expected. Taken as a whole, then, the data leave practically no escape from the conclusion that many, if not all, minute rearrangements are products of single ionizations (or at least of single activations). That is, both chromosome-breaks must be caused by the same electron hit, through some indirect, though localized, effect of the latter.

Since the meeting of the congress at which the above results were presented, a paper has appeared by Marshak (1939 *b*), which gives data showing a linear frequency-dosage relation for tiny fragments of chromosomes, cytologically detected in dividing cells of *Vicia* that had been irradiated during the preceding resting period. As these fragments appear to correspond with what is to be expected as a result of minute deletion, caused by two breaks very close together (although there is some doubt as to whether they are really small enough, and although Marshak for unexplained reasons regards them as mostly terminal), the linearity of their variation in frequency with change in dose probably provides evidence corroboratory of ours, and thus helps to confirm the above conclusion that the effects of single ionizations often spread in more than one

TABLE X

Frequency of mutations involving specific loci, in relation to dose of irradiation (combined results of five series)

Scheme of mating: Irradiated $sc^8 B \odot$ crossed with females of composition $y ac sc pn w v g f ci$. All mutations involving recessive alleles of $y ac pn w lz v g f B$ or ci , or dominant N , were found by inspection of $F_1 \odot \odot$, and gross deletions of X , giving g^+ , by inspection of $F_1 \odot \delta$. (Changes of sc , being too uncertain of recognition in comparisons with scute 8, are not recorded here.)

Dose in r.	No. of $F_1 \odot \odot$	Gene mutations and minute rearrangements*			Minute rearrangements†				Gross rearrangements			
		Alleles of w exclusive of u^{pm}		Total	Yellow	Notch revers.	Bar- revers.	Total	ci	Mottleds of X	Gross dele- tions	Total
		No. of $F_1 \odot \odot$	No. of v, g, f and lz		No.							
4000	22,330	19½	35	54½	73½	11	25	109½	71	18	29	118
1000	69,396	20½	32	52½	68½	14½	17	100½	30	7	14	51
0 (control)	20,051	1	1½	2½	2½	0	0	2½	0	0	0	0
												0.073
												0.0

* Between a third and a fourth of these mutations, at all the loci named, acted as recessive lethals, and most of these were probably minute deletions.

† Probably including some gross rearrangements, more especially in the case of B^+ .

[Fractional values are caused by the inclusion of fractional (mosaic) mutants, which however constituted but a small proportion of the total number of mutants.]

TABLE XI

Total number of induced mutations found for the low dose, compared with that expected, as calculated from frequency found for the high dose on various assumptions as to the value of x , where frequency varies as (dose) x

	No. calculated for low dose, when		No. found at low dose*
	$x=1.0$	$x=1.5$	
Gross rearrangements	91.7 ± 8.4	46.0 ± 4.2	23.0 ± 2.1
Minute rearrangements	83.1 ± 8.5	41.5 ± 4.3	20.7 ± 2.1
"Gene mutations"	40.6 ± 5.9	20.3 ± 3.0	10.1 ± 1.5
			44.0 ± 8.7

* Corrected by subtracting those calculated to be of spontaneous occurrence.

direction, though for a very limited distance, so as to cause two nearby breaks at once.¹

(b) *The delay between breakage and union*

If we had had minute rearrangements alone to study, our genetic results would not have disclosed to us the fact that breakage of the threads precedes their union. But, having found that this holds in the case of gross rearrangements, economy of assumptions recommends that we regard it as probable, provisionally at least, for the minute rearrangements as well. According to this view, then, they are formed by a mechanism intermediate between that operating on the extreme contact and the extreme breakage scheme. For a proximity of the two parts to be exchanged is the first condition, and the two breakages are then produced by a common exciting cause, that is, the same ionization, but this cause operates first to bring about their breakages, and fusion of the broken ends follows later—no doubt after fertilization, in the case of irradiated spermatozoa.

That this is true in at least some cases of minute rearrangements is further indicated by the fact that minute and gross rearrangements are sometimes intimately combined, in such a way as to show that one of the broken ends formed by two breaks very near together had united with a broken end formed by a distant break. Examples of this phenomenon are the author's minute deletion-insertion associated with scute-19 (1935), Oliver's (1937) probable mutual exchange of the facet locus in the *X* with a large interstitial piece of the third chromosome, and some reported minute interstitial "deficiencies" adjoining large inversions or trans-

¹ It may be noted in passing that the thinness of these fragments as compared with unbroken chromatids does not, as Marshak supposes, bear witness to the fragments being composed of "half-chromatids", but merely to the fact that their contained chromonemas are too short to form a succession of coils of the dimensions of those present in the ordinary undivided chromatids. In the latter, the apparent thickness really represents the diameter of the coils and, as Darlington has pointed out on various occasions, there is a false appearance of doubleness, arising from the greater conspicuousness of the two edges of a semi-transparent helix than of its middle part.

In the *Records of the Genetics Society of America* for 1939 (no. 8, p. 132), which has just come to hand since the above was written, there is a paper by C. M. Rick ("X-ray induced chromosome deletions in relation to mutation rate in *Tradescantia*") reporting that small ring-shaped fragments produced from chromosomes of *Tradescantia* by irradiation follow an exponential frequency-dosage relation. As this would indicate that these fragments arise in the manner of gross rearrangements, as a result of two independent breaks, the question is raised (1) as to why they tend to be so small, and (2) as to why these results and Marshak's on small fragments disagree. The answer to (1) might be connected with an effect of proximity of broken ends on their chances of union with one another.

locations. In these cases, then, the broken ends destined for the minute rearrangements must certainly have delayed their union until the same time (after fertilization) as that at which the union of broken ends destined for gross rearrangements took place. It is admitted, however, that further data, of a statistical character, are needed to determine to what extent the breaks involved in minute rearrangements are ordinarily available for simultaneous gross rearrangement in this manner. Present evidence is enough to indicate strongly that ends derived from breaks so near together are much more apt to unite with one another than with other ends, simultaneously present, derived from breaks farther away.

In considering the delay of union of ends formed by minute rearrangement, the question also arises of to what extent these unions take place between the broken ends of unsplit chromosomes or, alternatively, between the broken ends of the chromatids formed, subsequently to breakage, by the division of the spermatozoon chromosomes. That some minute rearrangements arise in the latter way is indicated by the presence of "fractional" (mosaic) mutants among them, for "fractionals" are nearly all chromatid changes, though not necessarily involving exchanges between the two sister chromatids. However, fractionals form not more than about a fifth of mutants in general (after irradiation of sperm) and minute rearrangements are no exception in this respect. Moreover, the scarcity of losses of chromosomes caused by union of sister broken ends (§ IV (*d*)) provides further evidence that the unions of broken ends formed by irradiation of sperm usually precede chromosome division.

It occurred to the writer some years ago that opportunity to obtain further light on the above question is provided by the fact that in the case of ring chromosomes chromatid rearrangements involving an exchange of connexions between sister strands must lead to double dicentric chromosomes, which are lost. This would necessarily be true no matter how minute these rearrangements were. Now since so large a proportion of lethals induced by X-rays in spermatozoa really consist of minute rearrangements (mostly deletions), there should be a marked reduction in the frequency of sex-linked lethals inducible in sperm containing ring chromosomes, as compared with non-rings, if a considerable proportion of minute rearrangements involve chromatid exchanges. Pursuant of this question, an experiment of this type was carried through by Offermann in 1936, and he has kindly provided me with a summary of his results for publication here (see Table XII). It will be seen that there is no perceptible difference here between the results for the ring and non-ring X-chromosomes. Accordingly, the minute rearrangements induced by

irradiation of sperm must include comparatively few cases in which a broken end of one chromatid unites with a broken end of a sister chromatid.

TABLE XII

Lack of influence of ring conformation on frequency of lethals recovered after irradiation of spermatozoa (data of Offermann)

Irradiation was in all cases carried out on impregnated females, within 1 to 2 days after impregnation in series A, B and C, and 1 week after in series D.

Series	Ring X-chromosome (Xc^2)					Non-ring X-chromosome					Weight of series (w)
	No. of fertile cult. (n_1)	No. of lethals (l_1)	No. of semi-lethals (sl_1)	% of $l_1 + sl_1$ (p_1)	$p_1 \times w$	No. of fertile cult. (n_2)	No. of lethals (l_2)	No. of semi-lethals (sl_2)	% of $l_2 + sl_2$ (p_2)	$p_2 \times w$	
A	268	12	1	4.85	11.9	225	14	1	6.67	16.3	245
B	321	25	2	8.43	13.2	103	8	4	11.64	18.2	156
C	256	11	2	5.09	11.6	206	7	0	3.39	7.7	228
D	108	14	0	12.95	13.6	102	10	0	9.80	10.3	105
Sum	953	62	5	6.87*	50.3	636	39	5	7.29*	52.5	734

* Weighted mean % ($= \Sigma(pw)/\Sigma w$).

Difference between weighted mean % = $0.3\% \pm 1.3\%$ (its standard error†).

† The weighted mean percentages and the standard error of their difference have been calculated according to the formula given in connexion with Table I.

(c) *Bearings on the interpretation of "sensitive volume"*¹

The proof of the proposition that more than one break can be caused by one ionization (or other activation?) and that therefore the breaks can be initiated by ionizations taking place outside of the part in which the genetic effect is produced has as a corollary the warning that the size of the material undergoing a genetic change (in this case breakage) cannot be determined by studies of the frequency with which the change takes place, in relation to the frequency of the ionizations induced, as has been attempted by various investigators. This was pointed out by the author (1932) at the Sixth Genetics Congress, on first presenting evidence of the production of multiple genetic effects by one ionization, but the present definitive demonstration of this phenomenon now makes it desirable to emphasize this conclusion, as well as to explain in greater detail certain other criticisms which we have brought forward against this method (Muller, 1937).

The method in question, dating at least as far back as Crowther's (1924, 1926) calculations on the sensitive volume responsible for the death of irradiated cells, may best be explained here on the basis of an example

¹ This entire subsection was added subsequently to the reading of the author's paper at the Seventh International Genetics Congress.

42 *Structural Change in Chromosomes of Drosophila*

dealing with gene mutation, as worked out by Mott-Smith and the author in 1930 (unpublished, but mentioned in connexion with the warning above referred to (Muller, 1932)). Roughly one mutation from the normal allele to white eye or some other detectable allele of it is induced in 1000 spermatozoa of *Drosophila* by irradiation with 5000 r. (Muller, 1928), hence it takes something of the order of 5,000,000 r. definitely to induce one detectable mutation in the given gene in a given spermatozoon, on the average. With this dose, in material of atomic composition like that in protoplasm, there are roughly 10^{19} ionizations (formations of ion-pairs) per c.c., i.e. one ionization in 10^{-19} c.c. Some one of these ionizations in a sperm cell must be the effective one in producing the mutation in question, and since this ionization may involve any atom at random within a total volume of 10^{-19} c.c. of material (assuming that the sensitive material is of atomic make-up like that in protoplasm), we may (still on this assumption) conclude that any "hit" atom in this amount of material will act as a "trigger" for the mutation reaction.

The material in question would contain something of the order of 2700 atoms and, if gathered together into the form of a sphere, have a diameter slightly less than $\frac{1}{100} \mu$.¹ This, as it happens, is almost identical with the example later worked out in Blackwood's (1931, 1932) independent calculations, except that he used as data the lower mutation frequencies occurring in somatic cells, as found by Patterson (1929), and so reached a correspondingly lower estimate of the volume. It has been assumed by various authors that this "sensitive volume"—or one calculated by a method in some way analogous to this, though based on other data—represents the actual size of the gene itself, or, more generally, of the genetic material subject to the type of change in question.

It will be seen that this identification involves the following main assumptions: (1) that any ionization (or other activation?) of any atom within a gene unfailingly results in a genetic change, never being in-

¹ The "sensitive volume" calculated would not represent the actual volume or number of atoms capable of giving a mutation unless they were taken as being of weights apportioned similarly to those of protoplasm in general. If, for example, the initiating atoms were in large measure phosphorus atoms the volume would be correspondingly less, on account of the greater density of phosphorus and its disproportionately greater chance of ionization. Thus the "sensitive volume" only gives that volume, composed of protoplasm, which would have an ionizability equal to that of the hypothetical "sensitive volume" sought. It should also be noted that if, as seems more likely, an individual ion, no matter whether positive or negative, instead of an ion-pair or ion of one sign only, is the effective agent, the calculated volume becomes half as large. In these calculations, moreover, we disregard the effect of possible clustering of the ions (*vide infra*), which surely obtains so far as the two ions of a pair are concerned.

effective or followed by restitution, (2) that this genetic change is always a genetic change of the type being looked for and detectable by the method used, (3) that the ionization of no atom outside the gene can, through a transmission of energy, result in such a change of the gene. Unfortunately for the validity of the method as a means of finding gene or chromonema size, every one of these three assumptions is not merely gratuitous but improbable on theoretical grounds and there is in fact strong empirical evidence against each of them, in connexion with either breakages or gene mutations or both. More specifically, in the case of breakages, the finding that one ionization can result in more than one genetic change is definitive evidence against the third assumption, at least as applied to such cases.

Further evidence against the validity of the identification of the gene with the "sensitive volume" as calculated above is, as pointed out by Fricke & Demerec (1937), provided by their own and by Timofeeff-Ressovsky & Zimmer's (1935 *b*) findings that when, by the use of very long wave-length X-rays, the ions in the electron paths are crowded together so closely that two of them would often lie within the same "sensitive volume", as above calculated, nevertheless there is no reduction in the efficiency of the ionizations in producing mutations, as compared with the efficiency of more widely scattered ions. If each ion within a region of the given size would, singly, be an effective one, then a crowding of them in this way would deprive the "extra" ions of their chance of detectable effectiveness. The same thing may perhaps be illustrated more clearly by taking as an extreme illustration the supposition that the ions always came in clusters of ten, all of them in atoms directly adjacent to one another; in this case all ten at once would rarely be able to produce more than one detectable mutation, since they would all be in the same "sensitive volume" if in any at all, and so the mutation frequency would be only about 1/10 that produced by scattered ions. Thus the lack of effect of crowding shows that most of the ionizations are incapable of resulting in mutations, even when they occur within the so-called sensitive volume.

In recent work of Zimmer & Timofeeff-Ressovsky (1938), the very greatly crowded ions formed as a result of neutron irradiation (shown to produce mutations by Nagai & Locher, 1937, 1938, under guidance of Altenburg) have indeed seemed to be somewhat less effective than more scattered ions, but this result is as yet subject to considerable revision, because of the uncertainties of physical measurement (Zimmer, 1938). If substantiated, it would seem to point to a clustering of effective atoms in

sensitive subregions within the main "potential sensitive volume" (see below). For both the mutation frequency calculations and, when taken in connexion with them, the experiments with long wave-length X-rays, just cited, prove the potential sensitive volume as a whole to be larger than these neutron experiments, taken at their face value, would indicate.

The above empirical facts concerning the multiple effects of ionizations and concerning the comparative lack of influence of ion crowding, as well as other theoretical considerations, lead us then to infer, quite contrary to the assumptions under criticism, that (1) only a comparatively rare ionization, even when it occurs within a gene, leads to a mutation, and especially to a detectable one, and (2) ionizations outside the gene also can produce genetic changes, certainly those of the nature of breaks, and perhaps also gene mutations. The "sensitive volume" is thereby relegated to an expression for the number of atoms which at any given moment are so situated, and in such a condition as regards their configuration and dynamics, that their ionization, if produced according to some randomly specified pattern out of the various possible energy patterns for an electron "hit", will result in a mutation of the specified kind. Some of these atoms may be inside the gene and others outside, how far out we do not know. Moreover, there is no reason to suppose that the atoms in question are the same ones from moment to moment, as the conformation changes, since there may be many atoms which only under special circumstances will give a mutation when ionized. Thus a more intelligible picture would be that of a "potential sensitive volume", comprising the whole region within which an ionization might on one occasion or another result in a mutation, and to say that the "sensitive volume" as above calculated consists of this "potential sensitive volume", divided by the average chance that any atom in the latter would if ionized give rise to the mutation. This is the way in which Timofeeff-Ressovsky and his co-workers (1939), as well as the present author and his co-workers, look at the matter.¹

The determination of the average chance that an ionization shall be effective can hardly be made by present methods, however, unless it should turn out that the chance is large enough for the size of the "potential sensitive volume" itself to be estimated by some method in-

¹ In some earlier work (Timofeeff-Ressovsky & Zimmer, 1935b) it had been stated that "der empfindliche Bereich mit dem Gen zu identifizieren ist", but in discussions of the subject at the "gene group conferences" held at Copenhagen, Denmark, in September 1936, and at Spa, Belgium, in November 1938, both sets of workers were found really to hold substantially the above point of view with regard to it.

volving crowding of ions, like those above mentioned. Moreover, it is evident that either the average chance or else the actual size of the potential sensitive region can change greatly with variation in conditions, as shown by the marked differences in induced mutation frequency between spermatozoa and immature germ cells or larval somatic cells, and between the cells of sprouting seedlings and those of dormant seeds (Stadler, 1928)—facts which alone should have been enough to show that the “sensitive volume” cannot be identified with the gene.

Apart from the fallacies involved in the acceptance of the three assumptions mentioned above, other errors of calculation and technique invalidate much of the work hitherto done on sensitive volumes. For example, Gowen & Gay (1933), in calculating a value analogous to the “sensitive volume” for all mutations taken together (i.e. that for the sum of all genes) used the frequency of primary quantum absorptions instead of ionizations as representing the effective hits, thus introducing here an error of two orders of magnitude,¹ although in their own work on wavelength, in the same paper, they themselves showed (as previous workers had done) that the mutation frequency was proportional to total ionizations and not to the number of primary quanta absorbed. And in dividing this calculated figure for all genes taken together by the estimated total number of genes, in order to get the size of the individual gene, they used methods of calculation of gene number that were invalid on other grounds, as we have pointed out elsewhere (Muller, 1936 b).

Again, Marshak (1935, 1936, 1937, 1939 c), in an attempt to derive a value for chromonema size from its breakage frequency, instead of using the number of ionizations occurring in a region of given size, used the number of electron tracks which merely traversed it, regardless of whether they gave ionizations or not. Nevertheless, in his work on wavelength too it was shown that the number of ionizations or activations and not the number of primary quanta or total length of electron tracks (which, with shorter wave-lengths, is longer in proportion to the number of ionizations) was the factor determining the frequency of the effects studied (Marshak, 1937). For gene mutation, this bearing of wave-length studies had already been pointed out by Timofeeff-Ressovsky & Zimmer (1935 b). But there is, in fact, no known physical mechanism whereby

¹ The same method appears to be followed in Gowen's most recent estimate of virus size, which came to our hands after the above was written (*Proc. Gen. Soc. America*, 1939, and *Genetics*, 1940, 25, 118-19), inasmuch as the “length of the electron track” is stated to constitute one of the factors in the calculation. Moreover, the virus calculation in effect rests upon the same three gratuitous premises as the gene calculations above criticized.

the mere passage of the electrons, regardless of their effectiveness in producing ionizations, could be the determining factor in any given effect. Marshak assumed, moreover, that the effective electron did its work by traversing two adjacent chromonemas at once (on the contact hypothesis), but made no allowance in his calculations for the greater rarity of such a double event as compared with a single one. As a matter of fact, as we pointed out in some detail in connexion with our first criticism of "sensitive volume" work (Muller, 1932), the chance of the same electron giving an ionization or activation in two adjacent genes is negligible as compared with the chance of separate electrons affecting them, with the doses of radiation that must actually be used.

Finally, in the work of Haskins (1935) and Haskins & Enzmann (1936, 1938), designed to estimate the size of the gene at the locus of white-eye, following Blackwood, the genetic method used—irradiation of eosin-eyed flies—must have resulted in the erroneous classification of mutations at many different loci as mutations at the locus of white. Thus they based their calculations on a mutation frequency inordinately higher than in the accurate data of Patterson (1929), used by Blackwood (1931, 1932), and arrived at a "sensitive volume" far larger than that corresponding to the mutations of the white locus itself. And in view of the irreconcilable disparities between their method of calculation and Marshak's, it is strange to find them referring to the calculated results of the latter author as in agreement with theirs.

Because of all these sources of error, any apparent agreements between the "sensitive volumes" hitherto found and the maximum size of the gene as estimated by quite different methods must be regarded as purely fortuitous. Abandoning any such naïve identification of "sensitive volume" with gene or chromonema, it will in the future be of greater interest to attempt to throw light upon what atoms and atom configurations are the more important ones in the initiation of the mutation and breakage processes, and upon what kinds of steps may be involved in the secondary reactions occurring between the ionization and the genetic change itself.

VI. ULTRA-VIOLET TESTS: THE PROBLEM OF THE RELATION BETWEEN STRUCTURAL CHANGES AND GENE MUTATIONS

(a) *The dubiousness of the distinction between "intra-" and "extra-genic mutations"*

As was pointed out by Stadler (1932), and as had earlier been foreshadowed by Serebrovsky (1929) (as mentioned on p. 3), there is at present

no certain criterion for distinguishing structural changes, if very minute, from intra-genic mutations, and it is conceivable that all apparent gene mutations produced by irradiation are really "extra-genic" changes, in the sense of changes in the linear arrangement and number of genes, including both minute deletions and duplications, and minute rearrangements of other kinds, accompanied by position effects. This question becomes much more acute now, since our findings (Muller *et al.* 1934, 1935, 1937*a*; Muller, 1935; Belgovsky, 1938, 1939; Belgovsky & Muller, 1937; Raffel, 1938) that these minute structural changes, with their position effects, do in fact occur and are produced by radiation, that they may be of vanishingly small size, both from the cytological and from the genetic point of view, that they follow the same frequency-dosage curve as the supposed "gene mutations", and that they vary similarly in their incidence with the type of cell treated.¹

In fact, in *Drosophila*, the question may now be made even broader, to inquire whether the spontaneous gene mutations too—since they seem indistinguishable as a class from the gene mutations produced by radiation—may not be extremely minute variants of the structural changes (although in the plant material there has, as Stadler, 1932, has shown, been more reason for supposing that some of the spontaneous "gene mutations", as judged in some cases by their dominance and in others by the inappreciable influence of radiation upon the loci concerned, may belong to a different category or categories from the radiation mutations). It is not conceivable, however, that all spontaneous "gene mutations", forming, as they must, the essential building blocks for an indefinitely great amount of evolution, could consist merely of losses or changes in position of "whole genes", in the sense in which genes have hitherto been conceived, nor would this be consistent with a rational idea of how such a vast number of different genes had come to exist in the first place. It would therefore be necessary, if the linear rearrangement view of all spontaneous as well as induced mutations were adopted, to do away, largely, with the distinction between "extra-genic" and "intra-genic mutations". In that case, we could not postulate sharp boundaries between the regions denoted as genes, different from the boundaries between smaller subdivisions within them (such as, perhaps, the amino-acid radicals), and so we should have to admit the possibility of breaks and reunions between these smaller portions, i.e. within what had been considered single genes. This view—which I tentatively proposed some two years ago (1937) only as one possibility, suggested by the state of the

¹ In this connexion, see also the footnote on p. 26.

evidence then existing, but which Goldschmidt (1937 *a, b*, 1938) independently put forward at the same time and urged, in a series of suggestive papers, as the only reasonable interpretation, would seem to make of the "gene" only a rather artificially delimited region of the chromosome, without distinctive boundaries.¹

(b) *Ultra-violet as a possible means of discrimination.*

It is not feasible here to go into all the ins and outs raised by this question. But although the problem is ultimately a chemical one, it is possible that further genetic results may still throw light upon it. The main results so far which may prove to have a bearing on it are the proof by Stadler (1939) and Stadler & Uber (1938), following earlier indications by Stadler & Sprague (1936), that in maize ultra-violet light causes distinctly fewer structural changes (with the exception of terminal deficiencies) in relation to the frequency of the induced gene mutations, than does high-energy radiation (X- and γ -rays), and, more recently, the proof by Dr Mackenzie and myself (1939), following still earlier indications by Altenburg (1930, 1931, 1936), that in *Drosophila* there is no appreciable production of structural changes at all, by ultra-violet light which induces gene mutations at a fairly high frequency—high enough for them to have been accompanied by a quite appreciable frequency of structural changes if X- or γ -rays had been the agent. As shown by Table XIII, which gives a summary of our tests of this question up to the date of the congress (including data besides those previously published), the evidence for this conclusion is now much more than sufficient. If X-rays of the same gene-mutation producing strength had been used in these experiments instead of ultra-violet, some seventy-five translocations would have been observed, instead of none.

It is apparent, in the *Drosophila* material at any rate, that the ultra-violet light, though producing gene mutations, does so either without producing breaks in the chromosomes at all, or at least without producing thoroughgoing breaks, of the type produced by X-rays and by mechanical means. For, as we have seen previously, real breaks, when produced in

¹ While it may be seen from our § VI (c) that the present author does not go so far as Goldschmidt in rejecting the conception of genes as corresponding to natural segments of the chromosome, he believes Goldschmidt's insistence upon this possibility, and the bringing forward of the evidence against segmentalism, to constitute a needed step in genetic criticism. A recent summary of Goldschmidt's views on this question, to which the reader may be referred, is the paper "Chromosomes and genes", read at a symposium celebrating the Centenary of the cell theory in Stanford University, 2 July 1939. This paper was distributed in mimeographed form to those attending the gene group conference of the Seventh International Genetics Congress. Our § VI was written before we read this paper.

Drosophila spermatozoa, are later followed by fusions, and consequently by observable structural changes, such as are conspicuously absent after ultra-violet treatment. If we wished to bring the maize results into line with these, we might postulate that the terminal deficiencies (and rarer structural changes?) which are induced by ultra-violet in maize belong to a category more nearly akin to gene mutations, the primary effect being a transformation of an ordinary bipolar interstitial gene into a

TABLE XIII

Effect of ultra-violet light

Series	Tests for lethals			Tests for translocations	
	No. of fertile cultures	No. of lethals in <i>X</i>	% of lethals	No. of fertile cultures	No. of trans-locations
1	1070	22	2.1	2449	0
2	606	20	3.3	976	0
3	1375	64	4.6	1617	0
Sum	3051	106	3.7	5042	0

Scheme of matings: In series 1 and 2 *y w* males were irradiated.

For the lethal tests these were mated with *ClB* females and the *Bar* daughters bred in individual cultures, while for the translocation tests the *y w* males (irradiated at the same time as those for the lethal tests) were mated with *XX yellow brown ebony* females, their sons being back-crossed individually to females of the same kind. In series 3, males of composition *y sc⁴ w sc⁸* were irradiated and crossed to females of composition *sc dl49 v f dp e*. For the lethal tests daughters (*F*₁) from these matings were bred individually, the *F*₂ being examined for the presence of *y w* males. For the translocation tests sons (*F*₁) derived from the same matings (i.e. brothers of the *F*₁ females that were tested for lethals) were bred individually to *dp e* females and cases of lack of recombination of the markers, sex, *dp* and *e*, were looked for among the *F*₂ groups.

monopolar one (telomere). In that case the breaks caused in maize by ultra-violet, unlike those caused by X-rays, would only be the secondary products of changes more nearly resembling gene mutations.¹ And "gene mutation" itself might still be considered as arising, not merely by structural change of the linear rearrangement type, involving breakage

¹ McClintock's recent (1939) finding that in maize the change from interstitial gene to telomere may follow mere mechanical breakage, in cells of the sporophyte generation, shows that in this material the genes are in fact much more labile in their "polarity" or "genic valency" (i.e. in their tendency to unite with one or more other genes—see Kossikov & Muller, 1935) than they are in *Drosophila*. And although this lability is so great, and so subject to regulation, that these changes can perhaps be accounted as "mutations" only by an extension of the term, nevertheless this very lability strengthens our main thesis, according to which the terminal deficiencies produced by ultra-violet in plants were not caused by breakage of the usual kind, but were only secondary consequences of changes primarily induced in the "valency" of an individual gene. The fact that such changes in "valency" occur with so much greater difficulty, if at all, in *Drosophila*—i.e. that the telomere is so much more permanent an organ there—thus fits in with the apparent failure of ultra-violet to produce "simple breakages" in this form. And the basic conclusion would still hold, that ultra-violet, unlike X-rays, cannot cause primary breakage of the chromosomes (breakage that leaves both ends adhesive), either in the plant or animal material.

and reunion of single-file constituents on a minute scale, but also by chemical changes of other kinds. This then would still leave room for the older conception of definitely delimited genes, between which but not within which breakage by X-rays could occur, and within which but not between which changes by ultra-violet could occur. Possible physical reasons for such a difference in effect (such as the ionization of water caused by X-rays and not by ultra-violet, as suggested by Delbrück in a personal communication) would not be far to seek.

Whether such a view, or the alternative previously suggested, emerges as the more plausible one may depend upon whether or not ultra-violet is found to produce demonstrable minute rearrangements. That is a question upon which we are working at present, but extensive data seem to be necessary for answering it. A Notch, possibly a minute deletion, was once obtained in an ultra-violet experiment of Altenburg's in 1931 (reported in a personal communication), but this may have been an accident unrelated to the treatment. If it should be found that minute rearrangements are in fact produced by ultra-violet, then it would be evident that for the production of these minute rearrangements it is not necessary to have thoroughgoing chromosome breakage—like that produced by X-rays—that is, breakage that persists for a considerable time and eventually involves also the chromosome "sheath" and so allows the production of gross rearrangements. Yet even the minute rearrangements would require some sort of breakages. Conceivably these might be followed by fusion more readily, and within the unbroken "sheath". In that case it would be much more likely that apparent "gene mutations" too could be produced in such a manner. And this in turn would raise the presumption that the gene mutations too might be linear rearrangements, but of parts smaller than the putative "genes", the latter thus losing their distinct identities.

If, on the other hand, the ultra-violet were found not to give rise, like X-rays, to demonstrable minute rearrangements, such a restriction of the process of gene mutations to change in the linear order of genetic materials already present would seem as yet uncalled for, and the door would be left wider open to distinguish between gene mutations and gene rearrangements, and so to methods of, as it were, demarking the gene. Ultra-violet mutations might then be considered intra-genic and X-ray breaks intergenic, and it might be that the genic boundaries so established coincided with boundaries established in other ways, as by points where crossing-over was possible and by the limits of certain kinds of functional activities of the genes.

(c) *Other evidence regarding chromosome segmentation*

One line of evidence indicating a segmental structure of some sort in the chromonema is provided by my findings of the restricted number of the genetically distinguishable positions of breakage producible by X-rays in the neighbourhood of the loci of scute and yellow in *Drosophila* (Muller & Prokofyeva, 1934). The only plausible escape from the conclusion that there is a rather coarse segmental structure here is to suppose that between these "genes"—or minute chromosome regions whose functions we are observing—there lie longer stretches of genetic material, having so little or so unimportant functions that their total loss would still leave the organism viable and normal-appearing. In that case the visible "genes" would give the appearance of being discontinuous only because they were separated by long interspaces of relatively inert genic material and so the discontinuity would in a sense be of a functional nature only.

In this connexion a reason may be mentioned for inferring that the thoroughgoing chromosome breaks at least, those of a kind capable of giving rise to gross structural changes, probably do not break ordinary chemical bonds like the bonds between amino-acid radicals, but only bonds (if any) of indifferent sign, like the bonds between two carbon atoms. This reason lies in the fact that the broken ends cannot be classified into "plus" and "minus", since identical broken ends of sister chromatids are also capable of uniting with each other. This bespeaks a specialized structure for the places of breakage, that is, again, a segmental structure for the chromonema, constituted of segments larger than the amino-acid radicals.

Another line of evidence in favour of a segmental structure of the chromonema, not finer than would be set by the lengths of protein molecules (if indeed the gene consists of proteins) lies in a consideration of the facts of crossing-over. The principle of interference and the manner of its decrease with distance indicate that crossing-over is caused by a mechanical stress which breaks the chromatids, as has been pointed out by the present author (1916) and as has been more especially supported by further facts and arguments brought forward by Darlington (1932). This conception of crossing-over is now made still more probable by the proof, herein provided, that a part of this supposed mechanism—namely, breakage first, and then union of broken ends—actually does operate when structural change occurs (even though the cause of the breakage itself is not the same here, and cannot lead to breakage at homologous

points). Now it seems very unlikely that the mere mechanical tension of the chromonema which breaks the chromosome at crossing-over would reach such a pitch as to be able to break the chemical bond between two amino-acid radicals connected in the ordinary manner within a protein molecule, or to break any similar intramolecular linkage in the chain of a molecule of the type of protein, fat or lipid. It is much more likely, therefore, that there are special connexions, of a looser kind, between larger segments, and that it is at these "internodes" that crossing-over can take place. Moreover, it is difficult to conceive that the breakage would take place by mechanical means with such absolute precision as it does, at quite identical points in the homologous chromatids, if the possible breakage points were so numerous and closely crowded as, say, the links between the members of polypeptide or polysaccharide chains.

At the same time we must be careful not to take it for granted that the chromonemal internodes demarcated by different criteria would necessarily coincide with one another. X-ray breakage, for example, might be possible at more points than crossing-over breakage, or vice versa, and the points of crossing-over in turn might be different from the limits set by ultra-violet mutation or from the boundaries pertaining to certain types of gene-functioning, and there might be still different limits prescribed for the smallest amount capable of exerting auto-synthesis or auto-attraction. If then the different criteria gave different results we should have correspondingly different kinds of "genes", according to the definition of gene which we chose to follow. In fact, according to some criteria of their functioning genes are already known to occupy overlapping areas, in that they show the so-called position effect.

Thus we should beware of taking too simplified a view of the problems at issue. There may not merely be "wheels", but "wheels within wheels", in living matter, and if the recent studies of viruses by the X-ray diffraction method, as reported for instance by Astbury (1939) and by Crowfoot (1939) at the Seventh Genetics Congress, may be used as showing what structures might be found in the genetic material, according to the author's (1926) conception of viruses as representing relatively free genes, then it is not unlikely that there are various grades of division and subdivision, each with its special kind of arrangement, between the relatively gross bodies hitherto recognized by the biologist and the very much finer ones hitherto studied by the chemist. We cannot pretend to have answered such problems yet. They are, so far as geneticists are concerned, for the future, while for the present it is a step even to be able to recognize their existence. But that cannot, in my opinion, invalidate the

fundamentals thus far established of what has been called "gene theory", even though many geneticists have in the past adopted a too hard and fast if not arbitrary conception of how much constitutes one gene. In this work of the future, it is to be hoped, the techniques of chemistry and physics will increasingly take part, along with those of genetics.

VII. ON THE MECHANISM OF RADIATION NECROSIS

(a) *Genetic influences*

Before closing, a brief reference should be made to possible bearings of some of the above facts on some aspects of the problem of how death of cells and tissues exposed to X-ray and radium radiation comes about. We know that such radiation produces structural changes, gross and minute, and gene mutations. We know that many of these changes are detrimental to life in all sorts of ways, and special studies, too intricate to detail here, have shown that many of them retard the proliferation and even result in the death of individual somatic or germ cells in which they have occurred, or groups of cells derived from the latter. It is therefore to be inferred that the necrosis observed by radiologists is in part at least due to these genetic changes (see discussion by Scott, 1937).

When spermatozoa are exposed to radiation, these chromosomal and gene changes do not affect the vitality or functioning of the spermatozoa themselves, for the genes are inactive in them (Muller & Settles, 1927), but they can cause the death of the fertilized eggs which have received them, in comparatively early stages of their development. These particular lethal effects may thus be said to be dominant. The question may then be raised, are the deaths of all these embryonic tissues, containing previously irradiated nuclei, or portions of nuclei, to be ascribed to these dominant genetic changes, of the above three classes?

Calculations which I made in 1927 of the frequencies of "dominant lethal" mutations occurring in individual chromosomes (based on the extent to which the sex ratio was affected) indicated that they were not abundant enough to cause all these deaths (Muller, 1927), and although there was an error here caused by our ignorance of the existence of inert regions the correction of that error still leaves the main conclusion the same. I calculated at that time that if genetic changes accounted for all the deaths many of these changes must not be conceived as confined to individual chromosomes, but must involve more than one chromosome at a time. We now know that such cases—which we may now identify as aneupentric translocations—are in fact relatively abundant at the doses

used. When, in addition to the changes in individual chromosomes, we take the frequency of these changes into account, and the manner in which we now know their frequency to vary with dosage, we find that these known sources of genetic change taken together should probably be sufficient to account for the death of all the eggs that were killed by the radiation of the sperm.

The matter is too complicated and has too many unknowns for such a calculation as yet to be more than an extremely rough one, but the results are of the right order of magnitude, and their manner of variation when the dosage is altered is also of the type expected. It is therefore reasonable to assume at present that all these deaths of embryos derived from treated sperm are caused by genetic changes of known types. And it is to be observed that, with increase in the dose used, the gross structural changes, as opposed to the minute rearrangements and gene mutations, tend to become the predominating factor in the causation of the deaths, because of the exponential rise in frequency of the former changes with increase in dose, accompanied by a merely linear rise in frequency of the latter.

Although the above applies only to the cells of embryos derived from irradiated spermatozoa, the conclusion cannot be avoided that at least some of the cell deaths in directly irradiated tissues must be of the same origin. It is not unlikely, for example, that the apparent adhesiveness of their chromatids and chromosomes seen after irradiation is only an expression of the fusion of previously broken ends. Moreover, if such a transference of our conclusions derived from the irradiation of spermatozoa is valid, we can to some extent use our knowledge of the mechanism of production of the genetic changes to anticipate the results ensuing when the conditions of radiation, or of the tissue irradiated, are altered. For example, since in the ordinary somatic cell, unlike the mature spermatozoon, union of broken chromosome ends can probably occur, the time-intensity relation will play a role, as pointed out in § IV (*d*).

There are various reasons why, if the deaths are due to genetic changes, tissues undergoing active proliferation will be killed more easily than other tissues. For one thing, the formation of structurally changed chromosomes in them will sooner be followed by the loss of the acentric and dicentric fragments so formed, since these losses can occur only during mitosis. Secondly, the more active anabolism and, in general, the more active utilization of gene products which must occur in proliferating cells must result in a more rapid expression of the effects of their abnormal genetic constitution (including those of gene mutation) and so must lead

sooner to death or malfunctioning. Thirdly, proliferating tissues are less likely to be polyploid and thus partially protected from the effects of genetic changes of all kinds (see § VII (b)). Fourthly, with mitosis occurring more frequently, the broken chromosome ends derived from opposite sides of the same break are more likely to become moved apart before they can undergo restitutional unions, and in addition the chromosomes are more likely to divide between breakage and union, thus giving attached chromatid fragments. Fifthly, if chromosomes in mid-mitosis are more likely to be changed structurally by radiation, like the similarly condensed chromosomes in spermatozoa (as we might judge from work of Holthuzen, 1920, and others showing this period to be more sensitive to the lethal effects of radiation), then tissues containing more mitoses should be more harmed by radiation.¹

It may well be that the chromosomes in mid-mitosis, like those in spermatozoa, are more readily affected because the breaks or potential breaks are stored up in them, a circumstance which allows the broken ends to become moved apart before union occurs, and also allows all the breaks to be present in the cell at once, thus giving a greater chance for recombinations to occur. In this connexion it should be noted that some authors, on the contrary, have assumed that mitotic chromosomes were more resistant to being changed by radiation. This is because most of the structural effects are not yet observable at the time of the mitosis which is being subjected to the treatment. They should, however, be observable in the mitosis following this, and only after that in turn should the deaths due to structural changes occur.

The correlation of the facts now known concerning the mechanism of structural change, and the manner of occurrence of gene mutation, under the action of radiation, with the facts concerning the manner in which necrosis and malfunctioning of proliferating and other tissues is brought about by radiation, has only just begun. It would seem, however, to offer considerable promise of increasing our understanding and control of the necrotic processes.

(b) *Non-genetic influences*

At the same time, we should beware of explaining all possible results by the same formula. The phenomena dealt with in this paper are those resulting from irradiation of chromosomes, as effected in most nearly

¹ See for instance the recent paper of Guyer (1939, *Proc. Soc. exp. Biol. and Med.*, N. Y., **42**, 565-8) which has just come to hand. In this work cancer tissue was found to be more harmed by X-radiation when under the influence of colchicine.

isolated form in mature spermatozoa. That the irradiation of the whole cell may entail additional effects, of a quite different character, has been shown, among other things, by experiments in which eggs and embryos as a whole are irradiated. Eggs and young embryos tolerate a much lower dose than sperm, yet they do not have as many genetic changes induced in them. This in itself shows that much of the lethal action on them is non-genetic when the irradiation is applied to them directly. Now as Miss Lamy and the author (1939) have reported in a separate paper at the Seventh Congress, special studies of the relative viabilities of diploid and triploid embryos directly subjected to the same dosage of X-radiation show that the diploids are killed off at no higher rate than the triploids. This constitutes, in our opinion, cogent evidence from another angle that these deaths were not caused by structural changes or gene mutations in the chromosomes, for the diploids would not be as well protected as the triploids against the effects of genetic changes. These deaths, then, were caused by some disturbances of physiological processes that did not depend on any prior effects on chromosomes or genes.

We do not know to what extent such embryonic cells of *Drosophila*—doubtless still containing much yolk and other material that had been stored in the egg—may be representative of proliferating cells in general. But at least the results show that we should bear possibilities of this latter kind in mind, in addition to the possibilities of structural change and gene mutation, when searching for explanations of the necrotic processes accompanying irradiation. In so far as the deaths may be due to the genetic changes, the geneticist may be of help in the investigation and interpretation of the necrotic phenomena, but in so far as they are not he can only apply such tests as these, to obtain a negative verdict, and thus to show the need for the general physiologist as well.

SUMMARY

Data are presented which show that a given number of ionizations produced in *Drosophila* spermatozoa by irradiation result in the same number of gross structural changes regardless of (a) wave-length, from 50 kV. X-rays to γ -rays, (b) continuous dose or fractionation over a 3-week period, (c) fertilization immediately or 1 month after treatment, (d) radiation intensity, from 0.05 to 250 r./min., (e) temperature, from 5 to 37° C. Hence in spermatozoa undergoing irradiation, "primary effects" of individual ionizations accumulate independently until fertilization, and their *final* total number determines the number of structural changes produced.

These primary effects are not the genetically observed rearrangements themselves, but must combine, usually in twos, to produce the rearrangements as secondary effects, although not until after all of the primary changes have been produced (i.e. after fertilization). For the frequency of the genetically observed gross rearrangements varies as the $3/2$ power of the final (total) dosage, for the range from 1000 to 4000 r. This is (*vide* Stadler & Catcheside) the relation expected at those doses for *surviving* combinations of the primary changes, if the latter consisted of breakages or potential breakages. At lower doses, the observed exponent does not fall towards 1 (linearity), as it would if only a part of the rearrangements represented combinations of independent primary changes, but probably rises nearer to the square, as expected if all of them were formed in this way.

That the "primary changes" in the sperm are really breakages (actual or potential) is further indicated by data on whole chromosome losses, which would be caused by "simple breakages" that had failed to undergo combination (except of sister fragments). For the frequency-dosage relation shown by these cases (which are but a small minority of the total) is found to be an approximately linear one, as expected. It is inferred that XX, XO-type gynandromorphs and other whole-chromosome mosaics resulting from irradiation of spermatozoa represent cases in which union of broken ends was delayed until after chromosome division; restitution then occurred in one chromatid but not in the other, which consequently was lost. Similarly, chromosome mosaics in which the two first cleavage nuclei must have differed in regard to the structural changes they contained are probably derived not from the chromosomes having already been split in the treated spermatozoon stage, but from union of ends having been delayed until after chromosome division, and having then occurred differently in sister chromatids.

Additional evidence that breakage precedes union is provided by the multiple nature of some of the exchanges, and by data indicating that these multiple-exchange translocations (obtained from ring chromosomes) increase more rapidly than those having ordinary double exchanges, as the dose of radiation is increased. At all doses tested, however, the multiple-exchange cases were found to be more frequent than expected on a random distribution of breaks and unions; this result shows that initial proximity of broken ends favours their union, and that restitutions must therefore be favoured at the expense of all types of new combinations. Sidky's finding of a translocation between a non-radiated maternal and an irradiated paternal chromosome furnishes evidence from another angle that breakage precedes union.

Data are given showing that minute rearrangements of different kinds, unlike gross rearrangements, have a linear frequency-dosage relation. Hence their breaks are interdependent, caused by a spreading of the effects of the same ionization to neighbouring points on the chromonema spiral.

This spreading of effects provides decisive evidence against the assumption that the genetic effects of radiation necessarily result from the ionization of atoms which formed a part of the genetic material affected. For this and other reasons, pointed out in the text, it is fallacious to identify the so-called "sensitive volumes" or "sensitive areas", calculated from the frequency of induced genetic changes, with the actual sizes of the genes or chromonemal material (or viruses) affected.

It is calculated that gross and minute structural changes, together with dominant lethal gene mutations, are, together, of the order of frequency to explain the mortality found for cells (of fertilized eggs and embryos) derived from irradiated spermatozoa. A part of the necrosis produced by irradiation in proliferating tissue in general must be of the same origin, hence studies on the mechanism of structural change and gene mutation should help in the understanding of these effects. But this is not the only way in which irradiation kills such cells, as data (Lamy & Muller) on relative mortalities of irradiated triploid and diploid embryos show their death to be predominantly of non-genetic origin.

The finding that breakage precedes fusion in the process of structural change strengthens, by analogy, the case for the conception of breakage first as opposed to fusion first at the points of exchange of connexions in crossing-over, and this in turn favours the theory according to which the chromatids at the time of crossing-over are subject to a stress, which tends to break them mechanically.

The problems are discussed of whether "gene mutations" are only ultra-minute linear rearrangements and of whether the "gene" is not a sharply defined segment of the chromonema. Evidence is presented showing that ultra-violet produces gene mutations without first breaking the chromosomes and this result as well as others is regarded as raising difficulties for such a view. It is held, however, that this matter is as yet far from settled, and the situation may be more complex than is generally realized, as indicated by studies on viruses.

ACKNOWLEDGEMENTS

Thanks are hereby expressed to the Scottish Cancer Control Organization, and in particular to Dr J. J. M. Shaw, F.R.C.S., for the financial

support of these investigations, to the Medical Research Council for the provision of the radium, to Prof. A. J. Clark, of the Department of Pharmacology, University of Edinburgh, for his very helpful interest and for the loan of an X-ray dosimeter, to Dr W. C. Scott, of Imperial Chemical Industries, for invaluable aid and advice, to Dr C. A. Murison, of the Royal Infirmary, Edinburgh, for his co-operation in arranging the set-up for irradiation by the radium, and to Dr H. D. Griffith, of Marischal College, Aberdeen, for making measurements of the radium radiation. Grateful acknowledgement is also made to our co-workers at the Institute of Animal Genetics who participated in the respective portions of the work previously specified; these co-workers comprised: Dr K. Mackenzie, Dr S. P. Ray-chaudhuri, Dr A. R. Sidky, Dr J. K. Makhijani, Dr A. I. Makki, Miss R. Lamy, Mr G. Pontecorvo, Mr R. B. Singh, Dr P. Bhattacharya, and Miss H. Alexander. We also take pleasure in acknowledging our deep indebtedness to Prof. F. A. E. Crew for his encouragement and for providing the conditions and facilities which made the work possible.

REFERENCES

- ALIKHANIAN, S. I. (1937). "A study of the lethal mutations in the left end of the sex-chromosome in *Drosophila melanogaster*." *Zool. Zh.* **16**, 247-79. (Russ. with Eng. summary.)
- ALTENBURG, E. (1930). "The effect of ultra-violet radiation on mutation." (Abstr.) *Anat. Rec.* **47**, 383.
- (1931). "Genetic effects of ultra-violet radiation." (Abstr.) *Anat. Rec.* **51** (Suppl.), 108-9.
- (1936). "The production of mutations by the polar cap method of treatment." *Biol. Zh.* **5**, 27-34. (Eng. with Russian summary.)
- ASTBURY, W. T. (1939). "Protein and virus studies in relation to the problem of the gene." *Proc. 7th int. Cong. Genet.* (in the Press).
- BAUER, H. (1939). "Röntgeninduktion von Chromosomenmutationen bei *Drosophila*." *Proc. 7th int. Congr. Genet.* (in the Press).
- BAUER, H., DEMEREC, M. & KAUFMANN, B. P. (1938). "X-ray induced chromosomal alterations in *Drosophila melanogaster*." *Genetics*, **23**, 610-30.
- BELGOVSKY, M. L. (1937). "Dependence of translocation frequency in *Drosophila melanogaster* upon the X-ray dosage." *Trud. inst. Genet.* **11**, 93-124. (Russ. with Eng. summary.)
- (1938). "Influence of inert regions of chromosomes on the frequency of occurrence and type of changes in the adjacent active sections." *Bull. Acad. Sci. U.R.S.S.* (Otd. mat.-est., Ser. Biol.), pp. 1017-36. (Russ. with Eng. summary.)
- (1939). "Dependence of the frequency of minute chromosome rearrangements in *Drosophila melanogaster* upon X-ray dosage." *Bull. Acad. Sci. U.R.S.S.* (Otd. mat.-est., Ser. biol.), pp. 159-70. (Russ. with Eng. summary.)

60 *Structural Change in Chromosomes of Drosophila*

- BELGOVSKY, M. L. & MULLER, H. J. (1937). "Further evidence of the prevalence of minute rearrangement and absence of simple breakage in and near chromocentral regions, and its bearing on the mechanisms of mosaicism and rearrangement." (Abstr.) *Rec. Genet. Soc. Amer.* **6**; and *Genetics* (1938), **23**, 139-40.
- BLACKWOOD, O. (1931). "X-ray evidence as to the size of a gene." (Abstr.) *Phys. Rev.* **37**, 1698.
- (1932). "Further X-ray evidence as to the size of a gene; and as to the energy of mutation by ultra-violet rays." (Abstr.) *Phys. Rev.* **40**, 1034.
- BRIDGES, C. B. (1935*a*). "Salivary chromosome maps. With a key to the banding of the chromosomes of *Drosophila melanogaster*." *J. Hered.* **26**, 60-4.
- (1935*b*). "Cytological data on chromosome four of *Drosophila melanogaster*." *Trud. Dinam. Razvit.* **10**, 463-74. (Eng. with Russ. summary.)
- BUZZATI-TRAVERSO, A. (1939). "Direct proportionality between X-ray dosage and translocations between the 2 and 3 chromosomes in *D. melanogaster*." *Drosophila Information Service*, **11**, 43.
- CAMARA, A. (1939). "The effect of X-radiation on the chromosomes of *Aloe arborescens*." *Proc. 7th int. Congr. Genet.* (in the Press).
- CATCHESIDE, D. G. (1938*a*). "The effect of X-ray dosage upon the frequency of induced structural changes in the chromosomes of *Drosophila melanogaster*." *J. Genet.* **36**, 307-20.
- (1938*b*). "The bearing of the frequencies of X-ray induced interchanges in maize upon the mechanism of their induction." *J. Genet.* **36**, 321-8.
- (1939). "The mechanism of radiation-induced chromosome rearrangements." *Proc. 7th int. Congr. Genet.* (in the Press).
- CROWFOOT, D. (1939). "Recent work on the structure of crystalline proteins and viruses." *Proc. 7th int. Congr. Genet.* (in the Press).
- CROWTHER, J. A. (1924). "Some considerations relative to the action of X-rays on tissue cells." *Proc. roy. Soc. B*, **96**, 207-11.
- (1926). "The action of X-rays on *Colpidium colopoda*." *Proc. roy. Soc. B*, **100**, 390-404.
- DARLINGTON, C. D. (1932). *Recent Advances in Cytology*. With a foreword by J. B. S. Haldane. xviii+559 pp.; 2nd ed. 1937, xvi+671 pp. London: J. and A. Churchill Ltd.
- DEMEREK, M. (1939). "The nature of changes in the notch-white region of the X-chromosome of *Drosophila melanogaster*." *Proc. 7th int. Congr. Genet.* (in the Press).
- DEMIDOVA, Z. A. (1937). "Relation between the dosage of X-rays and the type of mutations in the X-chromosome of *Drosophila melanogaster*." *Bull. Biol. Med. exp. U.R.S.S.* **4**, 311-13.
- DUBININ, N. P. & KHVOSTOVA, V. V. (1935). "The mechanism of occurrence of complex chromosome rearrangements." *Biol. Zh.* **4**, 935-75. (Russ. and Eng. text.)
- FABERGÉ, A. C. (1939). "An experiment on chromosome fragmentation by X-rays in *Tradescantia*." *Proc. 7th int. Congr. Genet.* (in the Press).
- FRICKE, H. & DEMEREK, M. (1937). "The influence of wave-length on genetic effects of X-rays." *Proc. nat. Acad. Sci., Wash.*, **23**, 320-7.

- GOLDSCHMIDT, R. (1937*a*). "Spontaneous chromatin rearrangements in *Drosophila*." *Nature, Lond.*, **140**, 767.
- (1937*b*). "A remarkable parallelism." *Proc. Nat. Acad. Sci., Wash.*, **23**, 219-23.
- (1938). *Physiological Genetics*. ix + 375 pp. New York and London: McGraw-Hill Book Co., Inc.
- GOWEN, J. W. & GAY, E. H. (1933). "Gene number, kind, and size in *Drosophila*." *Genetics*, **18**, 1-31.
- HASKINS, C. P. (1935). "A determination of the magnitude of the cell 'sensitive volume' associated with the white-eye mutation in X-rayed *Drosophila*." *Proc. nat. Acad. Sci., Wash.*, **21**, 561-6.
- HASKINS, C. P. & ENZMANN, E. V. (1936). "A determination of the magnitude of the cell 'sensitive volume' associated with the white-eye mutation in X-rayed *Drosophila*. II." *Proc. nat. Acad. Sci., Wash.*, **22**, 397-400.
- (1938). "A determination of the magnitude of the cell 'sensitive volume' associated with the white-eye mutation in X-rayed *Drosophila*. III." *Proc. nat. Acad. Sci., Wash.*, **24**, 136-41.
- HEPTNER, M. A. & DEMIDOVA, Z. A. (1936). "The relation between the dosage of X-rays and the mutation of single genes in *Drosophila melanogaster*." *Biol. Zh.* **5**, 541-50. (Russ. with Eng. summary.)
- HOLTTHUZEN, H. (1920). "Beitrage zur Biologie der Strahlenwirkung." *Pflüg. Arch. ges. Physiol.* **187**, 1-24.
- KAUFMANN, B. P. (1939). "Distribution of induced breaks along the X-chromosome of *Drosophila melanogaster*." *Proc. 7th int. Congr. Genet.* (in the Press).
- KAUFMANN, B. P. & BATE, R. C. (1938). "An X-ray induced intercalary duplication in *Drosophila* involving union of sister chromatids." *Proc. nat. Acad. Sci., Wash.*, **24**, 368-71.
- KHEVOSTOVA, V. V. & GAVRILOVA, A. A. (1935). "A new method of detecting translocations." *Biol. Zh.* **4**, 905-16. (Russ. with Eng. summary.)
- (1938). "Relation between the number of translocations in *Drosophila melanogaster* and the X-ray dosage." *Biol. Zh.* **7**, 381-90. (Russ. with Eng. summary.)
- KIRSSANOW, B. A. (1937). "Der Entstehungsmechanismus der Translokationen bei *Drosophila melanogaster*." *Biol. Zh.* **6**, 845-906. (Russ. with German summary.)
- KOSSIKOV, K. V. & MULLER, H. J. (1935). "Invalidation of the genetic evidence for branched chromonemas in the case of the pale translocation in *Drosophila*." *J. Hered.* **26**, 305-17.
- LAMY, R. & MULLER, H. J. (1939). "Evidence of the non-genetic nature of the lethal effect of radiation on *Drosophila* embryos." *Proc. 7th int. Congr. Genet.* (in the Press).
- LEVITSKY, G. & ARARATIAN, G. A. (1931). "Transformations of chromosomes under the influence of X-rays." *Bull. appl. Bot.* **27**, 265-303. (Russ. and Eng. text.)
- LEVITSKY, G. & SIZOVA, M. (1935). "Further studies on regularities in chromosome transformations in *Crepis capillaris* induced by X-rays." *C.R. Acad. Sci. U.R.S.S.* **4**, 70-1.
- MCCLINTOCK, B. (1932). "A correlation of ring-shaped chromosomes with variegation in *Zea mays*." *Proc. nat. Acad. Sci., Wash.*, **18**, 677-81.

62 *Structural Change in Chromosomes of Drosophila*

- McCLINTOCK, B. (1938a). "The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases." *Res. Bull. Mo. agric. Exp. Sta.* no. 290, 48 pp.
- (1938b). "The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behaviour of ring-shaped chromosomes." *Genetics*, **23**, 315-76.
- (1939). "The behaviour in successive nuclear divisions of a chromosome broken at meiosis." *Proc. nat. Acad. Sci., Wash.*, **25**, 405-16.
- MACKENSEN, O. (1933). "A cytological study of short deficiencies in the X-chromosome of *Drosophila melanogaster*." (Abstr.) *Rec. Genet. Soc. Amer.* **2**; and *Amer. Nat.* (1934), **68**, 76.
- (1935). "Locating genes on salivary chromosomes. Cytogenetic methods demonstrated in determining position of genes on the X-chromosome of *Drosophila melanogaster*." *J. Hered.* **26**, 163-74.
- MARSHAK, A. (1935). "The sensitive-volume of the meiotic chromonemata of *Gasteria* as determined by irradiation with X-rays." *Proc. nat. Acad. Sci., Wash.*, **21**, 227-32.
- (1936). "The effect of X-rays on chromosomes in different stages of meiosis." *J. gen. Physiol.* **19**, 179-98.
- (1937). "The effect of X-rays on chromosomes in mitosis." *Proc. nat. Acad. Sci., Wash.*, **23**, 362-9.
- (1938). "Alteration of chromosome sensitivity to X-rays with NH_4OH ." *Proc. Soc. exp. Biol., N.Y.*, **38**, 705-13.
- (1939a). "Effects of fast neutrons on chromosomes in mitosis." *Proc. Soc. exp. Biol. and Med., N.Y.*, **41**, 176-80.
- (1939b). "The nature of chromosome division and the duration of the nuclear cycle." *Proc. nat. Acad. Sci., Wash.*, **25**, 502-10.
- (1939c). "A comparison of the sensitivity of mitotic and meiotic chromosomes of *Vicia faba* and its bearing on theories of crossing-over." *Proc. nat. Acad. Sci., Wash.*, **25**, 510-16.
- (1939d). "Chromosome structure in meiosis and mitosis with reference to the mechanism of crossing-over." *Proc. 7th int. Congr. Genet.* (in the Press).
- MATHER, K. & STONE, L. H. A. (1933). "The effect of X-radiation upon somatic chromosomes." *J. Genet.* **28**, 1-24.
- MEJVEDEV, N. N. (1935). "The contributory effect of cold with irradiation in the production of mutations." *C.R. Acad. Sci. U.R.S.S. N.S.* **4** (9), 283-5.
- (1938). "Contributory effect of heat with irradiation in the production of mutations." *C.R. Acad. Sci. U.R.S.S. N.S.* **19**, 301-2.
- MICKEY, G. H. (1937). "Effect of temperature on frequency of translocations produced by X-rays." (Abstr.) *Rec. Genet. Soc. Amer.* **6**; and *Genetics* (1938), **23**, 160.
- MULLER, H. J. (1916). "The mechanism of crossing-over. I-IV." *Amer. Nat.* **50**, 193-221, 284-305, 350-66, 421-34. (Revised in separate, 1916.)
- (1926). "The gene as the basis of life." *Proc. 4th int. Congr. Plant Sci. (Ithaca)* (1929), **1**, 897-921.
- (1927). "The problem of genic modification." *Verh. V int. Kongr. Vererbungs- u. Z. indukt. Abstamm.- u. Vererb. Lehre*, Suppl. I (1928), pp. 234-60.
- (1928). "The production of mutations by X-rays." *Proc. nat. Acad. Sci., Wash.*, **14**, 714-26.

- MULLER, H. J. (1932). "Further studies on the nature and causes of gene mutations." *Proc. 6th int. Congr. Genet. (Ithaca)*, **1**, 213-55.
- (1935). "The origination of chromatin deficiencies as minute deletions subject to insertion elsewhere." *Genetica*, **17**, 237-52.
- (1936*a*). "Über die Bestimmung des Verhältnisses der Mutationsrate zur Strahlendosis." *Strahlentherapie*, **55**, 72-6.
- (1936*b*). "The present (1935) status of the mutation theory." Read at de Vries Memorial Meeting, Leningrad, Nov. 1935. Publ. in Russian in *Priroda*, No. 6, 40-50 (1936) and in English in *Curr. Sci.*, Special No. March, 1938, pp. 4-15.
- (1937). "The biological effects of radiation, with especial reference to mutations." *Act. Sci. industr. no. 725, Reun. int. Phys. Chim. Biol.* **8**, 477-94.
- (1938). "The remaking of chromosomes." *Collect. Net, Woods Hole*, **13**, 181-95, 198.
- (1939). "The mechanism of structural change in chromosomes." *Proc. 7th int. Congr. Genet. (in the Press)*.
- (1940). "Bearings of the *Drosophila* work on systematics." *The New Systematics*, ed. by J. S. Huxley, Clarendon Press, Oxford (pp. 185-268).
- MULLER, H. J. & GERSHENSON, S. M. (1935). "Inert regions of chromosomes as the temporary products of individual genes." *Proc. nat. Acad. Sci., Wash.*, **21**, 69-75.
- MULLER, H. J. & MACKENZIE, K. (1939). "Discriminatory effect of ultra-violet rays on mutations." *Nature, Lond.*, **143**, 83-4.
- MULLER, H. J., MAKI, A. I. & SIDKY, A. R. (1939). "Gene rearrangement in relation to radiation dosage." Address to Genetical Society, London, December 1938. Publ. in *J. Genet.* **37**, no. 3.
- MULLER, H. J. & PROKOFYEVA, A. A. (1934). "Continuity and discontinuity of the hereditary material." *Bull. Acad. Sci. U.R.S.S. N.S.* **4**, 74-83. (In Russian and English.) Reprinted in revised form under title: "The individual gene in relation to the chromomere and the chromosome." *Proc. nat. Acad. Sci., Wash.*, **21** (1935), 16-26.
- (1935). "The structure of the chromonema of the inert region of the X-chromosome of *Drosophila*." *C.R. Acad. Sci. U.R.S.S. N.S.* **1**, 658-60. (Russ. and Eng. text.)
- MULLER, H. J., PROKOFYEVA, A. A. & RAFFEL, D. (1934). "Apparent gene mutations due to the position-effect of minute gene rearrangements." (Abstr.) *Rec. Genet. Soc. Amer.* **3**; and *Amer. Nat.* **69** (1935), 72-3.
- (1935). "Minute intergenic rearrangement as a cause of apparent 'gene mutation'." *Nature, Lond.*, **135**, 253-5.
- MULLER, H. J., PROKOFYEVA-BELGOVSKAYA, A. A. & RAFFEL, D. (1937*a*). "The absence of transmissible chromosome fragments resulting from simple breakage, and their simulation as a result of compound breakage involving chromocentral regions." (Abstr.) *Rec. Genet. Soc. Amer.* **6**; and *Genetics* (1938), **23**, 161.
- MULLER, H. J., RAFFEL, D., GERSHENSON, S. M. & PROKOFYEVA-BELGOVSKAYA, A. A. (1937*b*). "A further analysis of loci in the so-called 'inert region' of the X-chromosome of *Drosophila*." *Genetics*, **22**, 87-93.
- MULLER, H. J. & SETTLES, F. (1927). "The non-functioning of the genes in spermatozoa." *Z. indukt. Abstamm.- u. Vererb. Lehre*, **43**, 285-312.

64 *Structural Change in Chromosomes of Drosophila*

- NAGAI, M. A. & LOCHER, G. L. (1937). "Production of mutations by neutrons." *Nature*, **140**, 111-12.
- (1938). "The production of mutations in *Drosophila* with neutron radiation." *Genetics*, **23**, 179-89.
- NAVASHIN, M. (1930). "Unbalanced somatic chromosomal variation in *Crepis*." *Univ. Calif. Publ. agric. Sci.* **6**, 95-106.
- OFFERMANN, C. A. (1936). "Branched chromosomes as symmetrical duplications." *J. Genet.* **32**, 103-16.
- OLIVER, C. P. (1930). "The effect of varying the duration of X-ray treatment upon the frequency of mutation." *Science*, **71**, 44-6.
- (1932). "An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations." *Z. indukt. Abstamm.- u. Vererb. Lehre*, **61**, 447-88.
- (1937). "Evidence indicating that facet in *Drosophila* is due to a deficiency." *Amer. Nat.* **71**, 560-6.
- PANTER, T. S. & MULLER, H. J. (1929). "Parallel cytology and genetics of induced translocations and deletions in *Drosophila*." *J. Hered.* **20**, 287-98.
- PAPALASHWILI, G. (1935). "The effect of a combined action of X-rays and low temperature on the frequency of translocations in *Drosophila melanogaster*." *Biol. Zh.* **4**, 587-91. (Russ. with Eng. summary.)
- PATERSON, J. T. (1929). "The production of mutations in somatic cells of *Drosophila melanogaster* by means of X-rays." *J. exp. Zool.* **53**, 327-72.
- (1931). "The production of gynandromorphs in *Drosophila melanogaster* by X-rays." *J. exp. Zool.* **60**, 173-211.
- (1932). "The mechanism of mosaic formation in *Drosophila*." *Proc. 6th int. Congr. Genet. (Ithaca)*, **2**, 153-5.
- (1933). "The mechanism of mosaic formation in *Drosophila*." *Genetics*, **18**, 32-52.
- (1935). "The question of delayed breakage in the chromosomes of *Drosophila*." *J. exp. Zool.* **70**, 233-42.
- (1938). "Sex differentiation. Aberrant forms in *Drosophila* and sex differentiation." *Amer. Nat.* **72**, 193-206.
- PROKOFYEVA-BELGOVSKAYA, A. A. (1937a). "Inert regions in distal ends of the chromosomes of *Drosophila melanogaster*." *Bull. Acad. Sci. U.R.S.S. (Otd. mat.-est., Ser. biol.)*, pp. 719-24. (Russ. with Eng. summary.)
- (1937b). "Observations on the structure of chromosomes in the salivary glands of *Drosophila melanogaster*." *Bull. Acad. Sci. U.R.S.S. (Otd. mat.-est., Ser. biol.)*, pp. 393-426. (Russ. with Eng. summary.)
- (1938). "The inert region in the subterminal part of the X-chromosome of *Drosophila melanogaster*." *Bull. Acad. Sci. U.R.S.S. (Otd. mat.-est., Ser. biol.)*, pp. 97-103. (Russ. with Eng. summary.)
- PROKOFYEVA-BELGOVSKAYA, A. A. & KHVOSTOVA, V. V. (1939). "Distribution of breaks in the X-chromosome of *Drosophila melanogaster*." *C.R. Acad. Sci. U.R.S.S.* **23**, 270-2.
- RAFFEL, D. (1938). "A genetic analysis of apparent losses of the distal end of the scute-8 chromosome." (Abstr.) *Rec. Genet. Soc. Amer.* **7**; and *Genetics*, **24**, 107.

- RAY-CHAUDHURI, S. P. (1939). "The validity of the Bunsen-Roscoe law in the production of mutations by radiation of extremely low intensity." *Proc. 7th int. Congr. Genet.* (in the Press).
- RHOADES, M. M. & MCCLINTOCK, B. (1935). "The cytogenetics of maize." *Bot. Rev.* **1**, 292-325.
- SACHAROV, V. V. (1935). "A cytological study of the lethals of the sex chromosome in *D. melanogaster*." *C.R. Acad. Sci. U.R.S.S. N.S.* **4** (9), 91-2.
- SAX, K. (1938). "Chromosome aberrations induced by X-rays." *Genetics*, **23**, 494-516.
- (1939). "The time factor in X-ray production of chromosome aberrations." *Proc. nat. Acad. Sci., Wash.*, **25**, 225-33.
- SAX, K. & ENZMANN, E. V. (1939). "The effect of temperature on X-ray induced chromosome aberrations." *Proc. nat. Acad. Sci., Wash.*, **25**, 397-405.
- SCOTT, C. M. (1937). "Some quantitative aspects of the biological action of X- and γ -rays." *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 223, 99 pp.
- SEREBROVSKY, A. S. (1929). "A general scheme for the origin of mutations." *Amer. Nat.* **63**, 374-8.
- SIDKY, A. R. (1939). "Translocation between sperm and egg chromosomes as evidence that breakage precedes union." *Proc. 7th int. Congr. Genet.* (in the Press).
- SLIZYNSKA, H. (1938). "Salivary chromosome analysis of the white-facet region of *Drosophila melanogaster*." *Genetics*, **23**, 291-9.
- SLIZYNSKI, B. M. (1938). "Salivary chromosome studies of lethals in *Drosophila melanogaster*." *Genetics*, **23**, 283-90.
- STADLER, L. J. (1928). "The rate of induced mutation in relation to dormancy, temperature, and dosage." (Abstr.) *Anat. Rec.* **41**, 97.
- (1932). "On the genetic nature of induced mutations in plants." *Proc. 6th int. Congr. Genet. (Ithaca)*, **1**, 274-94.
- (1936). "The nature of mutations. II." *Collect. Net, Woods Hole*, **11**, 248-51.
- (1939). "Genetic studies with ultra-violet radiation." *Proc. 7th int. Congr. Genet.* (in the Press).
- STADLER, L. J. & SPRAGUE, G. F. (1936). "Genetic effects of ultra-violet radiation in maize. I. Unfiltered radiation. II. Filtered radiations. III. Effects of nearly monochromatic $\lambda 2537$, and comparison of effects of X-ray and ultra-violet treatment." *Proc. nat. Acad. Sci., Wash.*, **22**, 572-8, 579-83, 584-91.
- STADLER, L. J. & UBER, F. M. (1938). "Preliminary data on genetic effects of monochromatic ultra-violet radiation in maize." (Abstr.) *Genetics*, **23**, 171.
- TIMOFEEFF-RESSOVSKY, N. W. (1932). "Mutations of the gene in different directions." *Proc. 6th int. Congr. Genet. (Ithaca)*, **1**, 308-30.
- (1939). "The mechanism of point mutations." *Proc. 7th int. Congr. Genet.* (in the Press).
- TIMOFEEFF-RESSOVSKY, N. W. & ZIMMER, K. G. (1935a). "Strahlengenetische Zeitfaktorversuche an *Drosophila melanogaster*." *Strahlentherapie*, **53**, 134-8.
- (1935b). "Wellenlängenunabhängigkeit der mutationsauslösenden Wirkung der Röntgen- und Gammastrahlung bei *Drosophila melanogaster*." *Strahlentherapie*, **54**, 265-78.
- (1938). "Neutronenbestrahlungsversuche zur Mutationsauslösung an *Drosophila melanogaster*." *Naturwissenschaften*, **26**, 362-5.
- Journ. of Genetics XL

66 *Structural Change in Chromosomes of Drosophila*

- TIMOFEEFF-RESSOVSKY, N. W. & ZIMMER, K. G. (1939). "Mutationsauslösung durch Röntgenbestrahlung unter verschiedener Temperatur bei *Drosophila melanogaster*." *Biol. Zbl.* **59**, 358-62.
- ZIMMER, K. G. (1935). "Über die Natur der Genmutation und der Genstruktur. Zweiter Teil: Die Treffertheorie und ihre Beziehung zur Mutationsauslösung." *Nachr. Ges. Wiss. Göttingen, Math.-phys. Kl., Biol. N.F.* **1**, 217-23.
- (1938). "Dosimetrische und strahlenbiologische Versuche mit schnellen Neutronen. I." *Strahlentherapie*, **63**, 517-27.
- ZIMMER, K. G. & TIMOFEEFF-RESSOVSKY, N. W. (1938). "Dosimetrische und strahlenbiologische Versuche mit schnellen Neutronen. II." *Strahlentherapie*, **63**, 528-36.

THE HETEROPYCNOSIS OF SEX CHROMOSOMES AND ITS INTERPRETATION IN TERMS OF SPIRAL STRUCTURE

BY M. J. D. WHITE

*University College, London and Department of Zoology,
Columbia University, New York City*

(With Plates I-III and Nine Text-figures)

1. INTRODUCTION

THE term heteropycnosis was introduced to describe the differential thickening of some chromosomes which takes place during mitosis and meiosis; heteropycnotic chromosomes thicken to a greater or less extent than the rest of the chromosome set. Heitz (1933) and others have used the term *heterochromatin* (as opposed to *euchromatin*) to describe chromatin which exhibits the property of heteropycnosis. The distinction seems to imply a constant chemical difference which is assumed to exist between the two kinds of chromatin; but we do not know whether this difference resides in the protein part of the "chromatin" or in the nucleic acid which is associated with it.

Heteropycnosis is particularly well seen in many sex chromosomes, but is not confined to them, being also found in many autosomes or parts of autosomes. It is at least possible that all chromosomes contain short heteropycnotic regions, and that the difference between heteropycnotic and non-heteropycnotic chromosomes is merely one of degree, depending on the number and length of the heteropycnotic segments present.

In view of the amount of work which has been carried out in recent years on the spiral structure of chromosomes (Darlington, 1935; Kuwada & Nakamura, 1934; Huskins & Smith, 1935; Nebel, 1932; Matsuura, 1937; Oura, 1936) it is rather surprising that the relationship between heteropycnosis and spiral structure has not been investigated until now. The suggestion has even been made (Muller & Prokofieva, 1935) that whereas all non-heteropycnotic regions have a spiral structure the heteropycnotic regions are not spiralized at all. The present work was planned in order to discover in what way, if any, heteropycnosis could be interpreted in terms of spiral structure. The chromosomes of the long-horned

and short-horned grasshoppers (Tettigonidae and Acrididae) were found to be particularly suitable for an investigation along these lines, since the sex chromosomes show well-marked heteropycnosis and under certain circumstances their spiral structure can be seen very clearly (see Pls. I-III).

The extent to which the manifestation of heteropycnosis depends on conditions external to the chromosome is shown by the fact that very frequently the same chromosome will show heteropycnosis in one sex, but not in the other, or in some, but not in all the tissues of the body. Thus the *X*-chromosome of the Orthoptera Saltatoria shows very strongly marked heteropycnosis during the prophase of the first meiotic division in the male; in the female, however, the two *X*'s do not show any heteropycnosis at this stage (Mohr, 1915; McNabb, 1928).¹ This fact naturally suggested that the *X* underwent heteropycnosis in the male because it was unpaired. Several lines of evidence, however, show that this is not so. In the first place, unpaired *X*-chromosomes do not usually show any heteropycnosis in somatic divisions; secondly, many autosomal regions which are present in the diploid state show strong heteropycnosis during meiosis. Lastly, when two *X*-chromosomes are present in a tetraploid spermatocyte they still show heteropycnosis (White, 1933).

Heteropycnosis is thus best regarded as a reaction on the part of the chromosome to a special state of its immediate environment, namely, the nuclear sap. Under certain conditions the reaction of "heterochromatin" and "euchromatin" is the same, since they thicken to the same extent—under other conditions the difference between the two manifests itself. Unfortunately we as yet know nothing of the nature of the "special state" which is necessary for the development of heteropycnosis.

An association of heteropycnosis with genetical "inertness" has been found in *Drosophila* and in the B-type chromosomes of maize (Randolph, 1928). Whether all heteropycnotic chromosomes are genetically inert is not known, but appears probable. If so, sex chromosomes which are heteropycnotic along their whole, or almost their whole, length may be expected to carry very few genes.

Heteropycnosis may show itself in two ways, which have been called by Darlington (1937) *under-condensation* and *over-condensation*. The present author (White, 1935) has used the terms *negative* and *positive*

¹ The fact that the *X* does not manifest heteropycnosis in the homogametic sex is interesting for the following reason: if it did so chiasma formation between the two *X*'s would presumably be prevented, as it is in tetraploid spermatocytes (White, 1933). The absence of heteropycnosis in the *X*'s of the female is thus an adaptation which permits them to form a bivalent.

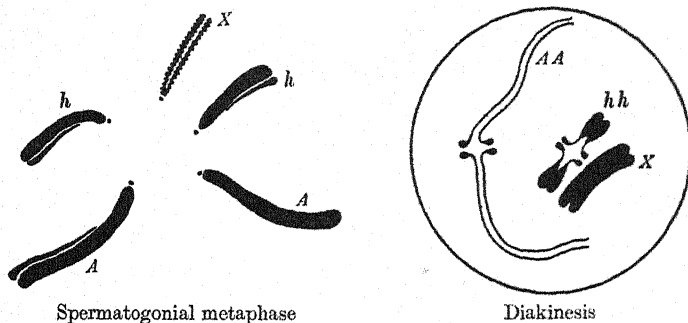
heteropycnosis in the same sense. A negatively heteropycnotic chromosome is one which thickens more slowly or to a lesser extent than the others during prophase or which undergoes de-condensation more rapidly during anaphase. Conversely, a positively heteropycnotic chromosome is one which thickens faster, earlier or to a greater extent than the rest of the chromosome set, or which remains thickened when the others are undergoing de-condensation at telophase.

It is important to realize that the same chromosome may show positive and negative heteropycnosis at different stages in its cycle. Thus the *X*-chromosomes of the short-horned grasshoppers show negative heteropycnosis in the early spermatogonial divisions (Text-figs. 1-4). The extent of negative heteropycnosis diminishes with the successive spermatogonial divisions, so that the *X* comes to behave more and more like an autosome, until at the last few pre-meiotic divisions there is no heteropycnosis, i.e. the thickening of the *X* is exactly similar to that of the autosomes in the same nucleus. After the last premeiotic division the *X* becomes positively heteropycnotic, so that in the prophase of the first meiotic division it is a thick sausage-shaped mass when the autosomes are still slender threads (Text-fig. 2*c*). At the metaphase of the first meiotic division the *X* and the autosomes are usually condensed to about the same extent; the *X* may even begin to show signs of negative heteropycnosis again (this usually shows itself at this stage as a slight roughening of the outline). During interkinesis the *X* may show positive heteropycnosis, and in the spermatids this is often very well marked (Text-fig. 9).

Cyclical reversibility is not, however, a feature shown by all heteropycnotic chromosomes. Thus in many Acrididae of the subfamily Truxalinae there exists one or more pairs of partially heteropycnotic autosomes (Janssens, 1924; Darlington, 1936; Carlson, 1936). In species of the genus *Mecostethus* there is a clear example of an autosome which shows strong positive heteropycnosis in its distal half at meiosis (McClung, 1927, 1928; White, 1937). This bivalent was called the "dyade compagnon" by Janssens, since it usually lies very close to the *X* during the prophase of the first meiotic division. In view of the parallel behaviour of the *X* and the distal part of the heteropycnotic autosome at meiosis it is rather surprising to find that at the early spermatogonial divisions, when the *X* shows negative heteropycnosis, the autosomal pair corresponding to the "dyade compagnon" shows no heteropycnosis of any kind (Text-fig. 1). As far as the Acrididae are concerned cyclical reversibility of heteropycnosis appear to be shown only by the *X*; heteropycnotic

autosomal regions only show positive heteropycnosis at the meiotic divisions.

In the crickets (Gryllidae) the X-chromosome in a number of species appears to show exactly the same type of reversible heteropycnosis as is found in the Acrididae (Ohmachi, 1935). In the Tettigonidae (long-



Text-fig. 1. Diagrams showing reversible and non-reversible heteropycnosis in *Mecostethus*. Only two pairs of autosomes and the X are shown. A = an ordinary autosome, h = the partially heteropycnotic autosome.

horned grasshoppers), on the other hand, the X shows positive heteropycnosis at the prophases and telophases of most or all the spermatogonial divisions as well as during the prophase of the first meiotic division: it never shows negative heteropycnosis at any stage in its cycle.

2. MATERIALS and METHODS

The following species of Orthoptera were used in the present investigation:

ACRIDIDAE.

Cyrtacanthacrinae (Catantopinae).

Melanoplus femur-rubrum De Geer (U.S.A.).

Schistocerca gregaria Forsk. (bred in the laboratory).

Oedipodinae.

Chorthippa viridifasciata De Geer (U.S.A.).

TETTIGONIDAE.

Decticinae.

Platycleis grisea Fabr. (England).

Metrioptera brachyptera L. (England).

Tettigoniinae.

Tettigonia viridissima L. (England).

Phaneropterinae.

Microcentrum sp. (probably *rhombifolium* Sauss.) (Mexico).*Insara tolteca* Sauss. (Mexico).*I. gracillima* Bruner (Mexico).*Leptophyes punctatissima* Bosc. (England).

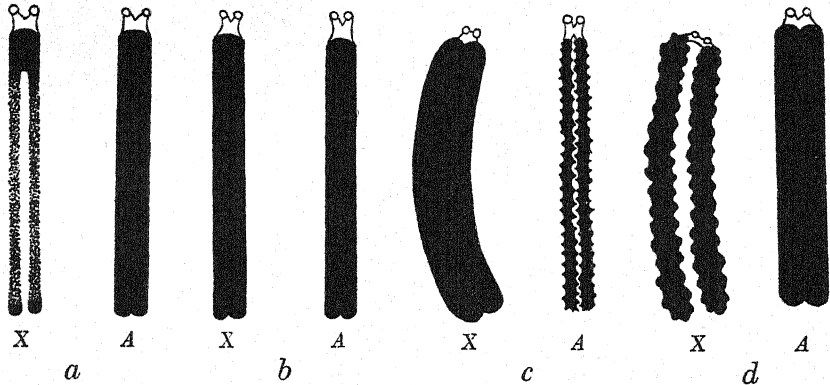
In most cases the testes were fixed in Flemming's strong fluid, cut at 15–20 μ and stained in gentian violet by Newton's method. In a few cases San Felice's fixative was used instead of Flemming. Since this is a very superior fixative for animal chromosomes and since it seems to be very little known it may be worth while to call attention to it:

Chromic acid 1%	16 parts
Commercial formalin	8 „
Glacial acetic acid	1 part

The ingredients should be freshly mixed just before use.

3. HETEROPYCNOSIS IN THE ACRIDIDAE

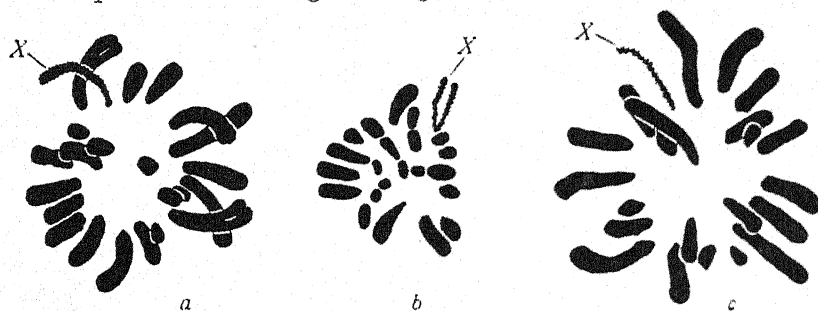
The negative heteropycnosis of the X-chromosome in the early spermatogonial divisions is shown by all the three species under consideration. It usually persists from early prophase to telophase, and is thus obvious throughout most of the mitotic cycle. At metaphase



Text-fig. 2. Diagrams illustrating the reversible heteropycnosis of the X in a short-horned grasshopper. *a*=an early spermatogonial metaphase, *b*=a late spermatogonial metaphase, *c*=diakinesis, *d*=first metaphase. In each case the X is shown alongside an autosome, so that the degree of thickening can be compared. In *c* and *d* the autosome is represented as a univalent.

(Text-fig. 3*a, b, c*) heteropycnosis manifests itself in three ways: (1) the X has an uneven outline, (2) the chromatids have a diameter which is considerably less than that of the autosomal chromatids, (3) in many

cases (e.g. Text-fig. 3*b*) it is possible to see the split between the chromatids much more clearly than in the autosomes. Many authors have stated that the X does not stain as deeply as the autosomes when it is negatively heteropycnotic; but it is possible that this is merely an optical effect due to the irregular outline. The spindle attachment is always "terminal" in these chromosomes, so that the two chromatids often appear to be united at the proximal end (Text-fig. 3*b*). Frequently the heteropycnosis is not quite uniform along the length of the chromosome; both the



Text-fig. 3. Early spermatogonial metaphases in (a) *Schistocerca*, (b) *Melanoplus* and (c) *Chortophaga*.

proximal and the distal ends (or only the former) may show less marked heteropycnosis than the middle region. An attempt to illustrate this diagrammatically has been made in Text-fig. 2 (which is mainly based on *Locusta migratoria*). The length of the X-chromosome at an early spermatogonial metaphase is given in Table I. These measurements are certainly not very accurate, but it is unlikely that the error is more than 10% of the total length.

TABLE I

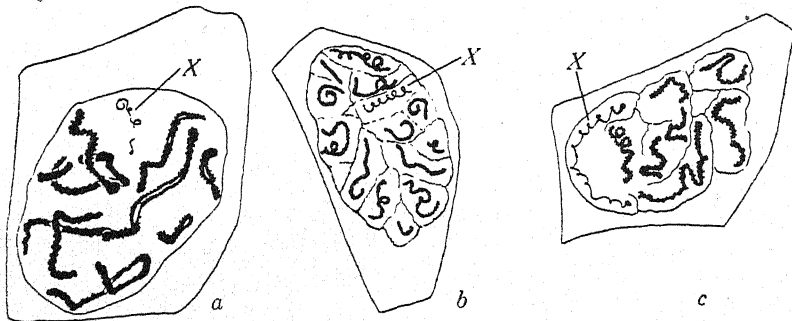
Length in micra of the X-chromosome in an early spermatogonial division and at diakinesis

Species	Spermatogonial metaphase	Diakinesis
<i>Schistocerca</i>	5.4	6.2
<i>Melanoplus</i>	3.3	3.3
<i>Chortophaga</i>	5.3	—

A special study was made of the X-chromosomes in the prophases of these spermatogonial divisions. At this stage it is usual for some or all of the chromosomes to show "relic" coils or spirals. These coils are to be regarded as the remains of the spirals of the previous division, whose gyres have now relaxed, and which are gradually uncoiling. Similar relic coils have been illustrated by Wenrich (1916) in the case of *Phryno-*

tettix; in the present material they can be seen very clearly in the case of the *X* (Text-fig. 4*a, b, c*). There can thus be no doubt that the negatively heteropycnotic *X* possesses a spiral structure at metaphase, although one has to wait until the succeeding prophase for direct visual evidence of its existence. Unfortunately, it is not possible to make an accurate comparison between the number of gyres in the *X* and the autosomes, but the number per unit length is probably higher in the *X*. Thus the *X*-chromosome in Text-fig. 4*c* would appear to have about twelve gyres, but it may have had more originally.

The main difference between the *X* and the autosomes lies, however, in the fact that the threads which form the spiral are much thinner in the case of the *X*. A negatively heteropycnotic chromosome is thus one



Text-fig. 4. Early spermatogonial prophase in (a) *Schistocerca*, (b) *Melanoplus*, (c) *Chortophaga*. The spiral structure of the negatively heteropycnotic *X* is visible. In each case only a few of the autosomes are drawn. In *b* and *c* the nucleus is vesiculate.

which fails to thicken during prophase to the same extent as the rest of the chromosome set. The failure to thicken does not, however, prevent it from assuming a spiral structure, i.e. thickening and spiralization are to some extent independent, and not merely two aspects of the same phenomenon.

It is clear that if we consider metaphase chromosomes as compact spirals whose gyres are in contact with one another, then the number of gyres per unit length will be inversely proportional to the thickness of the chromatid, that is to say, the thinner the chromosome the more gyres it will have per unit length. It is not clear, however, that negatively heteropycnotic chromosomes can be considered as "compact"—their outlines are too irregular and flocculent. It does, however, seem certain that the number of coils per unit length is greater in negatively heteropycnotic chromosomes, although it is possible that relation between

chromosome diameter and number of coils per unit length is not directly proportionate.

During the prophase of the first meiotic division the *X*-chromosome undergoes positive heteropycnosis: the details of this process have been described so many times that it is not intended to give a full account here, but merely to emphasize certain special points. During leptotene, zygotene and pachytene the *X* is a pear-shaped body, but is more or less "unfixable": that is to say that in fixed material it usually appears as a vacuolated body of very irregular shape which obviously bears little resemblance to the living structure.

After the end of pachytene the *X* becomes fixable and changes its appearance considerably. During diplotene and diakinesis it appears either as a single sausage-shaped body or (when the two chromatids are somewhat separated) as two sausages lying side by side. Its outline is smooth and its diameter much greater than in the case of the autosomal chromosomes which form bivalents at this stage. We may now speak of a true positive heteropycnosis uncomplicated by the special features which appear to be associated with it during leptotene-pachytene. It is not possible by ordinary methods to detect any spiral structure in the positively heteropycnotic chromosomes of diplotene-diakinesis; perhaps special techniques would reveal it, but they have not been tried as yet. The length of the *X* at diakinesis is shown in Table I—it will be seen that the length of the positively heteropycnotic *X* in meiosis is about the same as that of the negatively heteropycnotic *X* in a spermatogonial metaphase. To judge from the published figures the same relation appears to hold for other species. This shows in a very clear manner that heteropycnosis is not merely a matter of "differential contraction" as many workers appear to have supposed; it is due to differential thickening or lateral growth.

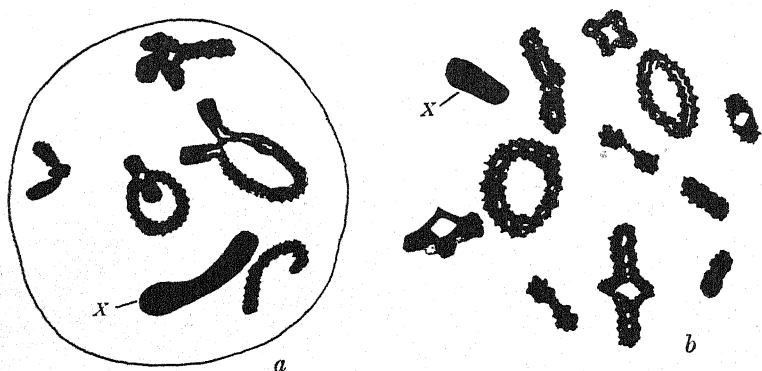
4. HETEROPYCNOSIS IN THE TETTIGONIDAE

In the long-horned grasshoppers we have to deal with only one type of heteropycnosis, namely, the positive kind. Very clear examples of spiral structure are found in some of the spermatogonial prophases. The relic spirals in the *X* have been figured by earlier writers (Mohr, 1916; de Winiwarter, 1931), but these authors did not study the direction of coiling, nor did they attempt to compare spiralization in the *X*-chromosome and the autosomes.

Of the seven species studied by me, *Leptophyes* has a very large *X*-chromosome with a submedian spindle attachment, while *Tettigonia*

has a V-shaped X-chromosome with a median spindle attachment. The other five species have rod-shaped X-chromosomes with a "polar granule" at the proximal end. *Platyleis* and the two species of *Insara* have 15 pairs of rod-shaped autosomes, *Microcentrum* has 16.

An excellent general account of the spermatogonial divisions in *Tettigonia* has been published by Mohr (1916); he referred to the species as *Locusta viridissima*—an incorrect designation which has led to some confusion in the literature, since there is a genus of short-horned grasshoppers called *Locusta*. Mohr emphasized the distinction between the primordial spermatogonia and the different generations of secondary spermatogonia, and he pointed out that in the latter there is no true resting stage, so that the distinction between the telophase of one division and the

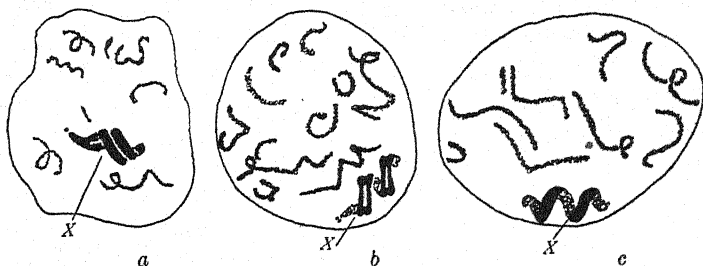


Text-fig. 5. Diakinesis in (a) *Schistocerca*, (b) *Melanoplus*.
a is from a section, b is from a smear.

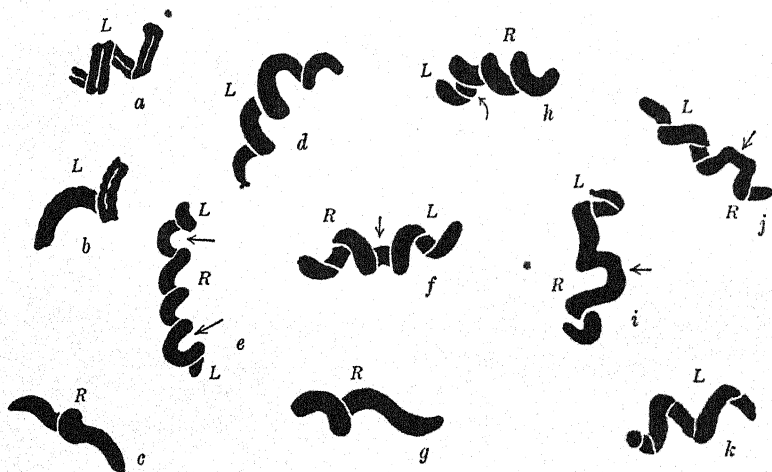
prophase of the next is an extremely arbitrary one. The general features of his description apply fairly well to all the Tettigonidae studied by me.

No heteropycnosis exists at metaphase in any of the spermatogonial divisions. The positive heteropycnosis of the X in the Tettigonidae is thus purely a prophase phenomenon. Three prophase nuclei (two of *Platyleis* and one of *Metrioptera*) have been drawn in Text-fig. 6; it will be seen that both the autosomes and the X show relic coiling, and that the threads which form the spiral are markedly thicker in the case of the X. Positive heteropycnosis is thus in this respect clearly the opposite of negative heteropycnosis as seen in the Acrididae. In some cases the split between the two chromatids can be seen quite plainly; they are jointly coiled at this stage. In Text-fig. 6a, b the "polar granules" at the proximal end of the X can be seen; they are also visible in Pl. I, fig. 6.

Pls. I, II and III consist of photomicrographs which show the spiral structure of the X in *Platycleis*, *Tettigonia* and *Insara*. In Pl. II, figs. 1, 2 and 3 (which are all taken from the same cyst) it is possible to compare the structure of the X and the autosomes. It should be pointed out that the tightness of the coiling is a very variable phenomenon in both X and autosomes. Thus in fig. 1 the X is very tightly coiled, while in figs. 2 and 3 the X's are relatively unwound, so that they are longer



Text-fig. 6. Spermatogonial prophases, *a* and *b* in *Platycleis*, *c* in *Metrioptera*.



Text-fig. 7. Eleven X-chromosomes from spermatogonial prophases in *Platycleis*. R and L indicate right- and left-handed coiling. Arrows indicate points of reversal of coiling.

and show fewer gyres. The X in the middle of fig. 3, in particular, is almost completely unwound.

In Text-fig. 7 the X-chromosomes of eleven nuclei in *Platycleis* have been drawn separately, showing various degrees of coiling. It will be noticed that the direction of spiralization (right-handed or left-handed) is not constant. In one individual of *Microcentrum* 41 "right-handed" and 30 "left-handed" X's were counted. These figures suggest that the

direction of spiralization is at random. In *Platypleura*, *Insara* and *Tettigonia* some cases were observed in which the direction of spiralization was reversed at an interstitial point, so that one end of the chromosome was "right-handed" while the other was "left-handed". In *Microcentrum* very few cases of reversal of coiling were found (three or four chromosomes in which an apparent reversal occurred have been omitted from the above totals).

It should be pointed out that these relic spirals are sufficiently clear for there to be no doubt in their interpretation; the only difficulty is when a reversal is believed to occur very near the end of a chromosome or when two reversals are believed to occur in the same chromosome, fairly close to one another (an event which has only been seen once or twice). Otherwise there is never any difficulty in determining whether a given chromosome is "right-handed" or "left-handed". Pl. I, figs. 1 and 2 show two X-chromosomes of *Tettigonia*; these same two chromosomes have been drawn in Text-fig. 8a, b. The first shows no



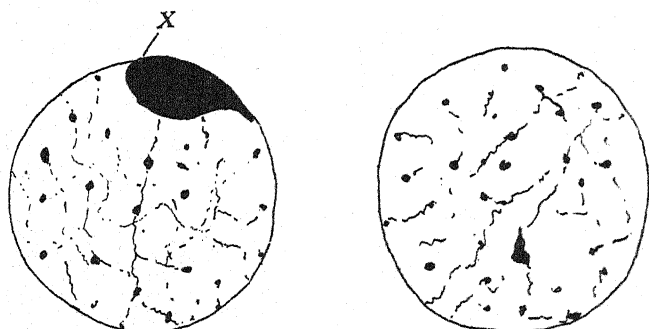
Text-fig. 8. a and b, two X-chromosomes from spermatogonial prophases in *Tettigonia*.

reversal of coiling, the second shows two points of reversal, one of which may coincide with the spindle attachment. It is necessary to point out that photographs frequently do not give a correct impression of the direction of coiling; if we consider a chromosome lying horizontally, an optical section above the mid-horizontal plane will give a true picture, while a photograph of an optical section below the mid-horizontal plane will give an impression of right-handed coiling where left-handed coiling is present and vice versa. This is because one involuntarily interprets everything which is out of focus as lying below the focal plane, while it may equally well lie above it. In the case of chromosomes which lie obliquely to the horizontal, a photograph may appear to show a reversal of coiling where none, in fact, is present; this is the case in Pl. I, fig. 1. When looking at actual preparations under the microscope one can focus up and down, so that one obtains a correct three-dimensional mental picture of the chromosome.

Pl. I, fig. 5 shows a telophase of a late spermatogonial division in *Tettigonia*. The X-chromosome is lying well apart from the mass of the autosomes and shows a large number of gyres which appear to have been

flattened against the nuclear membrane so that the chromosome is more nearly a zig-zag than a spiral. It will be noticed that the diameter of the zigzag is less in the middle of the chromosome (i.e. on either side of the spindle attachment) than at the ends; this is frequently the case at metaphase.

The behaviour of the X-chromosome during meiosis has been described for *Tettigonia* by Mohr (1916) and for *Decticus* by de Winiwarter (1931) and I have nothing to add to their accounts. The X appears to emerge from the premeiotic nucleus very much folded on itself; by a series of changes it finally reaches a sausage-shaped condition in diplotene-diakinesis, just as in the Acrididae.



Text-fig. 9. Spermatid nuclei in *Platycleis* with and without an X-chromosome.

5. DISCUSSION

The fact that one and the same chromosome may vary considerably in diameter from one division to another, while retaining essentially the same length, naturally suggests that the amount of nucleic acid varies from one stage to another. If we accept the view of Caspersson & Schultz (1938) according to which the protein framework of the chromosome is responsible for the synthesis of nucleic acid, then a negatively heteropycnotic chromosome is one in which the protein framework has synthesized less nucleic acid than is usually the case, while a positively heteropycnotic one has formed more nucleic acid. It is probable that a certain minimum quantity of nucleic acid must be present before the chromosome assumes a smooth outline—if less is present (as in a prophase chromosome or in a negatively heteropycnotic one at metaphase) the outline will be irregular and “flocculent”. On all modern views of chromosome structure the chromatids are regarded as being more tightly spiralized at metaphase than at any other stage. Now metaphase is presumably the time at which the amount of nucleic acid is also at

a maximum. It is therefore tempting to suggest that the assumption of the spiral form is a consequence of nucleic acid synthesis.

If we take the view that heteropycnosis is due to a special type of protein framework in the chromosome, then we must conclude that three types of protein framework are present in the chromosomes of the Acrididae: (1) that found in non-heteropycnotic autosomal regions, (2) that found in heteropycnotic autosomal regions, (3) that found in the *X*. Both the second and the third type give rise to heteropycnosis under appropriate conditions of the cellular environment, but the heteropycnosis due to the second type is non-reversible, while that due to the third is reversible. Some years ago the present author was inclined to believe that heteropycnotic autosomal regions had probably been derived from the *X* by translocation—but the fact that their heteropycnosis is of the non-reversible type seems to disprove this hypothesis and to suggest that they have developed *in situ*.

An interpretation of heteropycnosis which is entirely different from that expressed above has been put forward by Muller and his collaborators. These workers have suggested that there is no spiralization (or "less spiralization") of the heterochromatic regions of *Drosophila* chromosomes in somatic metaphases. At the same time Muller (1935) suggested that there may be as many as 250 coils in the "active" region of the *X* of *D. melanogaster*. These suggestions both appear highly unlikely in the light of the present work; they are based on a comparison between the salivary gland chromosomes and the mitotic *X*-chromosome—a comparison which is unjustified since it assumes that the chromonema does not stretch or contract to any extent.

To the present author it appears likely that all metaphase chromosomes are "compact" spirals (i.e. spirals with virtually no space in the axis and no space between the successive gyres). If this is so we may calculate the number of coils in any metaphase chromosome by dividing the length of the chromosome by half the diameter. Thus in the case of the *X*-chromosome of *D. melanogaster* (whose metaphase length is about 2μ and whose diameter is about 0.25μ), the number of gyres will be about $\frac{2}{0.125} = 16$. If one-third of the mitotic *X* represents the inert region this should include about 5–6 gyres, the "active" region having about 9–10. The exact number of gyres probably differs somewhat in different tissues: thus in the oogonia, where the *X* tapers somewhat towards the proximal end the number of gyres in the inert region is probably more than it is in some other tissues.

Various authors (Kuwada & Nakamura, 1934; Darlington, 1935; Oura, 1936) have described a "minor" spiral at meiosis in addition to the ordinary "major" spiral. One gyre of the major spiral is believed to consist of several gyres of the minor spiral. It might be suggested that the extra thickness of positively heteropycnotic chromosomes was due to their possessing a "minor" spiral in addition to the major one (the "control" chromosomes having only one spiral). Most of the published figures of the supposed minor spiral are too diagrammatic to constitute convincing evidence of its existence. Oura's Fig. 1, which is a photomicrograph of the first meiotic metaphase in *Tradescantia*, is in a different class, however. It has suffered considerably in reproduction, but the present author has seen a direct enlargement from the original negative which appears to show "minor" spirals very clearly. For the present, however, there seems no reason to complicate the interpretation of heteropycnosis by introducing a hypothetical minor spiral into the discussion, particularly since it has so far only been seen in plant chromosomes.

Various authors (Matsuura, 1935, 1937; Nebel, 1932) have produced evidence that the direction of spiralization is at random in plant chromosomes. The present work strongly confirms their results; it is particularly conclusive since only one chromosome has been considered, and its heteropycnosis has prevented the possibility of confusing it with any other chromosome.

I am greatly indebted to Prof. Franz Schrader for the facilities of his department at Columbia University during the winter of 1937-8. Most of the work in connexion with this paper was carried out in the U.S.A. during the tenure of a Rockefeller Fellowship by the author. The Mexican species were identified by Mr J. A. G. Rehn and Mr H. R. Roberts of the Academy of Natural Sciences, Philadelphia, to whom I am duly grateful.

REFERENCES

- CARLSON, J. G. (1936). "The intergeneric homology of an atypical euchromosome in several closely related Acridinae (order Orthoptera)." *J. Morph.* **59**, 123-61.
CASPARSSON, T. & SCHULTZ, J. (1938). "Nucleic acid metabolism of the chromosomes in relation to gene reproduction." *Nature, Lond.*, **142**, 294-5.
DARLINGTON, C. D. (1935). "The internal mechanics of the chromosomes. I, II and III." *Proc. roy. Soc. B*, **118**, 33-96.
— (1936). "Crossing-over and its mechanical relationships in *Chorthippus* and *Stauroderus*." *J. Genet.* **33**, 465-500.
— (1937). *Recent Advances in Cytology*, 2nd ed. London: Churchill.

- HEITZ, E. (1933). "Die somatische Heteropycnose bei *Drosophila melanogaster* und ihre genetische Bedeutung." *Z. Zellforsch.* **20**, 237-87.
- HUSKINS, C. L. & SMITH, S. G. (1935). "Meiotic chromosome structure in *Trillium erectum*." *Ann. Bot., Lond.*, **49**, 119-50.
- JANSSENS, F. A. (1924). "La chiasmotypie dans les insectes." *Cellule*, **34**, 135-359.
- KUWADA, Y. & NAKAMURA, K. (1934). "Behaviour of chromonemata in mitosis. II." *Cytologia, Tokyo*, **5**, 244-7.
- MATSUURA, H. (1935). "Chromosome studies on *Trillium kamschaticum* Pall. II." *J. Fac. Sci. Hokkaido Univ. Ser. 5*, **3**, 233-50.
- (1937). "Chromosome studies on *Trillium kamschaticum* Pall. IV." *Cytologia, Tokyo*, **8**, 178-94.
- MCCLUNG, C. E. (1927). "Synapsis and related phenomena in *Mecostethus* and *Leptysma*." *J. Morph.* **43**, 181-264.
- (1928). "Differential chromosomes of *Mecostethus gracilis*." *Z. Zellforsch.* **7**, 756-78.
- MCNABB, J. W. (1928). "A study of the chromosomes in meiosis, fertilization and cleavage in the grasshopper egg (Orthoptera)." *J. Morph.* **45**, 47-93.
- MOHR, O. L. (1915). "Sind die Heterochromosomen wahre Chromosomen? Untersuchungen über ihr Verhalten in der Oogenese von *Leptophyes punctatissima*." *Arch. Zellforsch.* **14**, 151-76.
- (1916). "Studien über die Chromatinreifung der männlichen Geschlechtszellen bei *Locusta viridissima*." *Arch. Biol.* **29**, 579-752.
- MULLER, H. J. (1935). "On the dimensions of chromosomes and genes in Dipteran salivary glands." *Am. Nat.* **69**, 405-11.
- MULLER, H. J. & PROKOFIEVA, A. A. (1935). "The structure of the chromonema of the inert region of the X-chromosome of *Drosophila*." *C.R. Acad. Sci. U.R.S.S. N.S.* **1**, 658-60.
- NEBEL, B. R. (1932). "Chromosome studies in the Tradescantiae. II. The direction of coiling of the chromonema in *Tradescantia reflexa* Raf., *T. virginiana* L., *Zebrina pendula* Schnitzl. and *Rhoeo discolor* Hance." *Z. Zellforsch.* **16**, 285-304.
- OHMACHI, F. (1935). "A comparative study of chromosome complements in the Gryllodea in relation to taxonomy." *Bull. Mie Coll. Agric. For.* no. 5.
- OURA, G. (1936). "A new method of unravelling the chromonema spirals." *Z. wiss. Mikr.* **53**, 36-7.
- RANDOLPH, L. F. (1928). "Chromosome numbers in *Zea Mays* L." *Mem. Cornell Univ. Exp. Sta.* no. 117.
- WENRICH, D. H. (1916). "The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes." *Bull. Mus. comp. Zool. Harv.* **60**, 57-135.
- WHITE, M. J. D. (1933). "Tetraploid spermatocytes in a Locust, *Schistocerca gregaria*." *Cytologia, Tokyo*, **5**, 135-9.
- (1935). "The effects of X-rays on mitosis in the spermatogonial divisions in *Locusta migratoria* L." *Proc. roy. Soc. B*, **119**, 61-84.
- (1937). "The effect of X-rays on the first meiotic division in three species of Orthoptera." *Proc. roy. Soc. B*, **124**, 183-96.
- DE WINIWARTER, H. (1931). "Évolution de l'hétérochromosome chez *Tettigonia (Decticus) albifrons* (Fab)." *Arch. Biol.* **42**, 201-28.

EXPLANATION OF PLATES I—III

PLATE I

Figs. 1, 2. *X*-chromosomes from spermatogonial prophases in *Tettigonia*.

Figs. 3, 4. *X*-chromosomes from spermatogonial prophases in *Platycleis*.

Fig. 5. Telophase of a late spermatogonial division in *Tettigonia*, showing the *X* lying alongside a compact mass of autosomes.

Fig. 6. *X*-chromosome from a spermatogonial prophase in *Insara*. This *X* shows the "polar granules" at the proximal end unusually clearly.

PLATE II

Figs. 1-3. General view of a cyst of spermatogonial cells in *Platycleis*, showing the spiral structure of *X*'s and autosomes. The *X* cannot be seen in all cells, since it is sometimes out of focus. Clear spiral structure can be seen in the autosomes labelled *A*.

PLATE III

General view of a cyst of spermatogonial cells in *Insara*. Only the *X*'s show spiral structure in this cyst, the structure of the autosomes being unclear. Five *X*'s can be seen in different parts of the photograph.

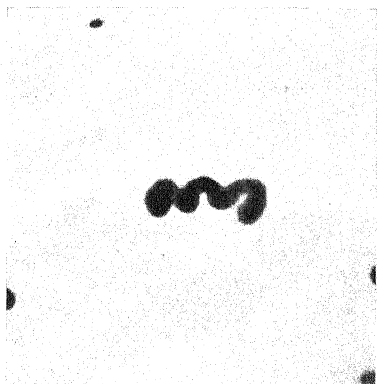


Fig. 1.



Fig. 2.

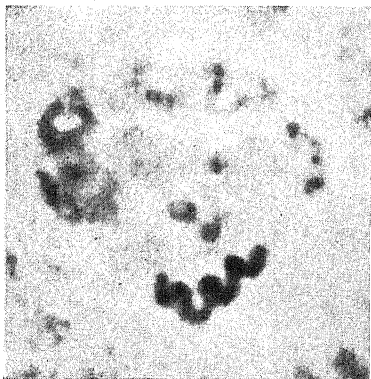


Fig. 3.

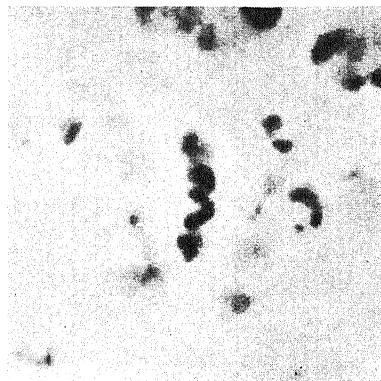


Fig. 4.

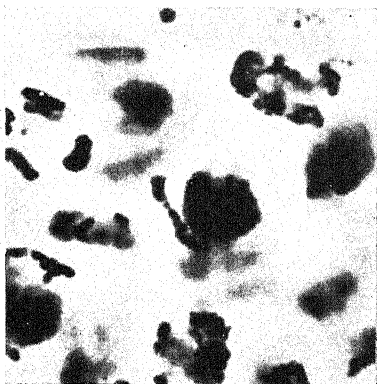


Fig. 5.

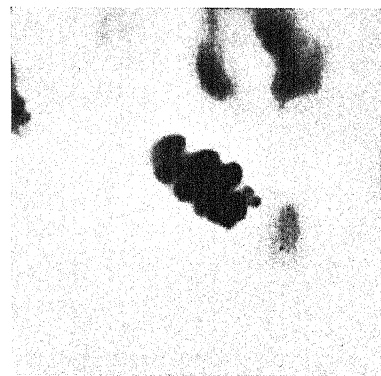


Fig. 6.

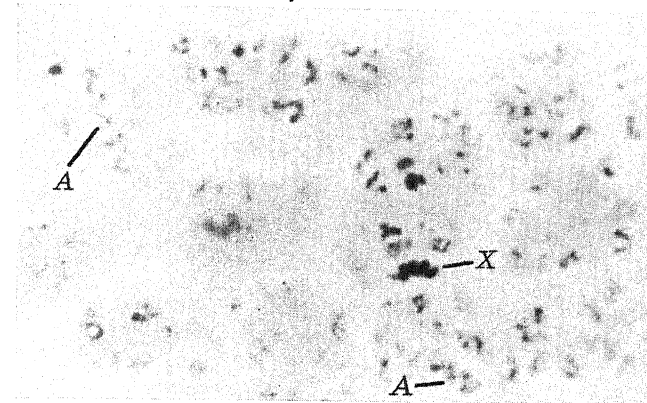


Fig. 1.

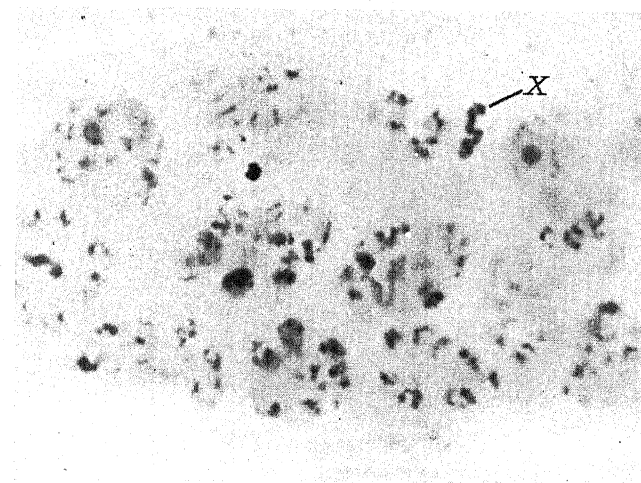


Fig. 2.

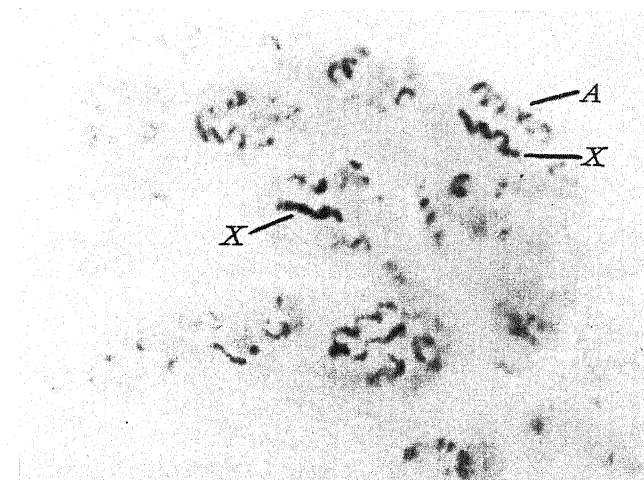
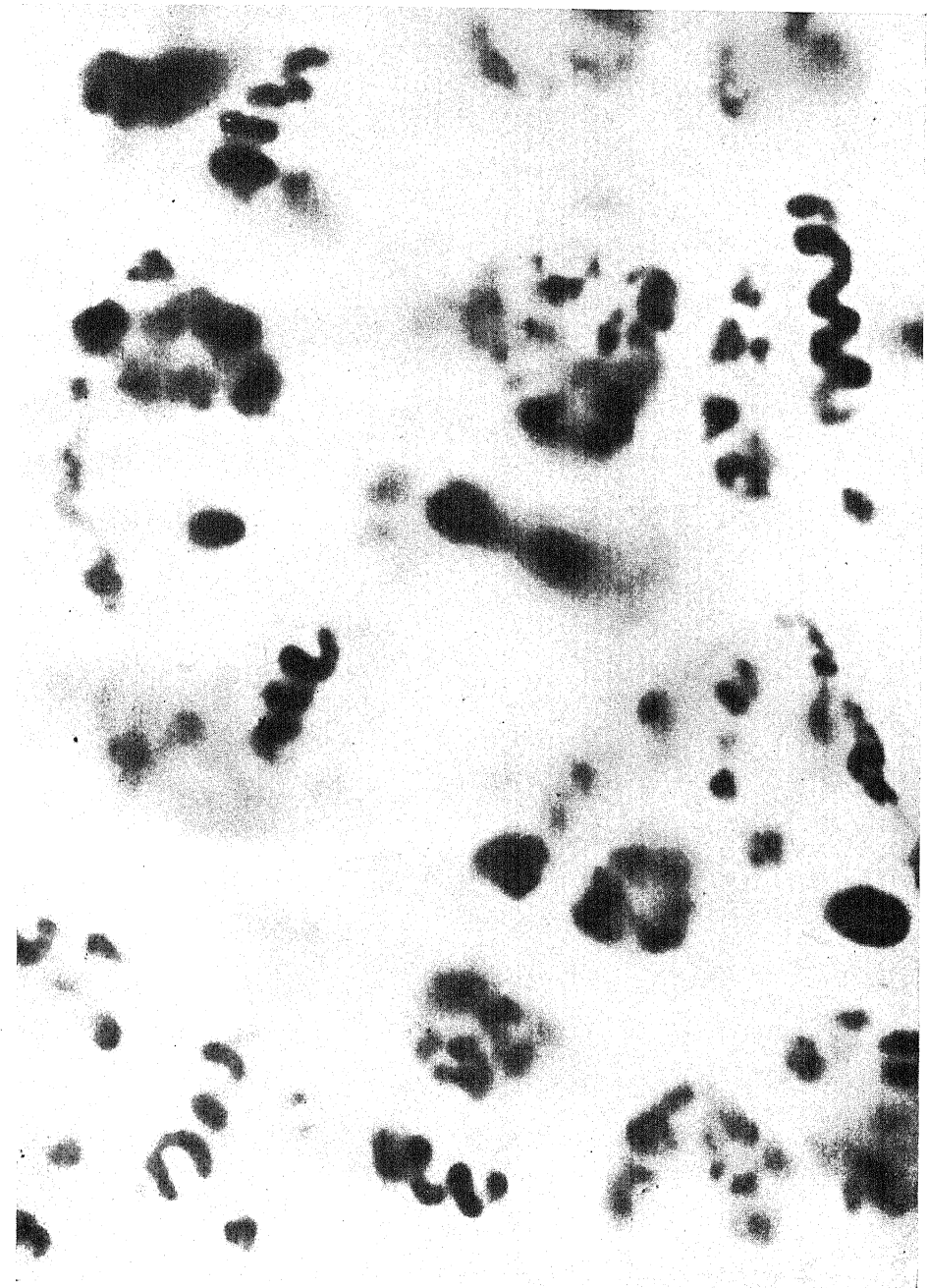


Fig. 3.



THE GENETICS OF *ARMADILLIDIUM* *VULGARE* LATR.

I. A GENERAL SURVEY OF THE PROBLEMS

By H. W. HOWARD

School of Agriculture, Cambridge

(With Plate IV and Five Text-figures)

CONTENTS

	PAGE
1. Introduction	83
2. Life history	84
3. Cytology	86
4. Breeding and feeding techniques	86
5. Genetics of the varieties	88
(a) Varieties <i>plumbeus</i> Lereb. and <i>variegatus</i> Lereb.	88
(b) Red varieties	89
(c) Type C	90
(d) Type D	93
(e) Var. <i>cooperi</i> Collinge	93
(f) Other types	94
(g) Summary and discussion	94
6. Sex ratios	96
(a) Terms used	96
(b) Summary of Vandel's work on <i>Trichoniscus</i>	97
(c) General considerations of monogeny in <i>Armadillidium</i>	98
(d) Frequency of occurrence of amphogenic and monogenic broods	100
(e) The inheritance of monogeny	102
(f) Discussion on monogeny	104
7. Analysis of natural populations	105
8. Summary	106
References	107
Explanation of Plate IV	108

1. INTRODUCTION

WOODLICE of the genus *Armadillidium* may be easily recognized by their habit of rolling up into a ball when disturbed—hence also their common name “pill bugs”. This habit makes them very convenient material to handle when scoring. There is no need to anaesthetize them. This fact suggested to the author that such woodlice would therefore be very useful material for amateur genetical research. In addition it was thought possible that they would not need much attention, e.g. they would only

need feeding every two weeks. Also they are small animals and so do not take up much room.

The first few populations examined contained in addition to the common black and greyish animals rarer varieties. A start was therefore made in examining the genetics of the various types. There was, however, soon found another interesting problem in the peculiar sex ratios found in many broods. Some broods consist entirely of females, others contain only males.

This paper contains (a) general notes on the suitability of *Armadillidium* for genetical research, (b) details of the breeding technique adopted, (c) results on the genetics of several of the varieties, (d) preliminary observations on the sex ratios found in different broods, (e) an analysis of a few wild populations, and finally (f) a general discussion on the problems suggested by this initial work.

2. LIFE HISTORY

In England *A. vulgare* has one brood per year, though a few animals produce two. The animals copulate in the spring and early summer. Some time after copulation the females shed their eggs into a brood pouch on the underside of their thorax (see Text-fig. 2). The eggs remain in this brood pouch until the young animals hatch. The broods of young animals are born from May onwards when the animals are kept in the laboratory. The incubation period is between two and three months. The fact that the females carry their eggs about with them is very useful, because it means that there is no risk of contaminating stocks by introducing eggs with the food. It is also useful in that several females can be kept with the same male and only separated from each other when found to be pregnant.

If the animal produces two broods in the same year, only one copulation is needed for both broods. I have also obtained evidence that sperms can live from one year to the next (see Table I). It is very unlikely that the three red animals in brood 25 were contaminants introduced with the food because they were also of the rareish type C. Red is dominant to

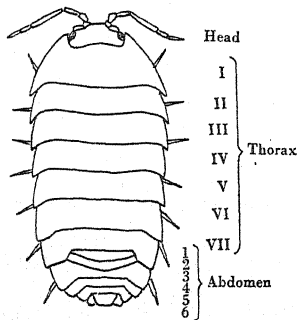
TABLE I

Brood 8	Born 1938	♀ AB (black, type C) × ♂ B (red)	16 black : 16 red
Brood 25	Born 1939	♀ AB (black, type C) × ♂ AM (black)	16 black, type B : 23 black, type C : 3 red, type C

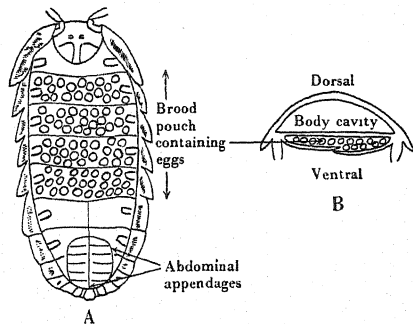
black (see later), and so at least three animals in brood 25 came from eggs fertilized by sperm a year old. It is not known definitely whether a

whole brood can be produced from sperm stored from one year to another, but in 1939 female H produced a brood of 52 animals very early in the year (animals born on 9 March or earlier). This female had been kept separate from any males for a long time and had not been placed with any male because it was so early in the spring. The brood may, of course, have been parthenogenetic.

In other animals, however, parthenogenesis has been found not to occur. Eight virgin females have been isolated from any males for more than two years. In their second year three of these animals produced brood pouches in which eggs could be seen. The eggs, however, in all three cases failed to develop.



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Outline drawing of dorsal view of *A. vulgare*.

Text-fig. 2. Pregnant female showing position of brood pouch. A. Ventral view, legs cut off at base. B. Diagrammatic section of a thoracic segment.

The number of animals per brood varies from about 50 to 150. An exceptionally large brood of 267 animals has been obtained from one female. The young animals are completely white when born and only develop their black and other pigments later. The amount of pigment developed increases from moult to moult, and it seems possible that *Armadillidium* might be useful for studies on the rate of action of genes (see also later in discussion of sex-limited varieties C and D).

If the animals are well fed, they can be scored for sex, colour, and colour patterns about two to four months after their birth. Also if well fed they become sexually mature in the spring following their birth. It is not known how many years they live, but broods can be obtained from females in both their first and second years. Males also live at least two years and are sexually potent in both these years.

In Toulouse (France) *A. vulgare* normally produces three broods per year (see Vandel, 1939 a). I have obtained some animals from Prof. Vandel, and it will be interesting to see whether, under the same laboratory conditions as the English animals, the Toulouse woodlice still produce three broods per year. My general impression is that under laboratory conditions English animals produce broods earlier in the year than they do in the wild. Environment may therefore control to some extent the number of broods produced per year.

3. CYTOLOGY

The chromosome number of *A. vulgare* has been found by Radu (1930) to be $n=27$ and $2n=54$ from examination of males. The chromosomes are unfortunately very small. Radu was not able to observe whether the male was heterozygous for the sex chromosomes. It seems unlikely, both from the large number of the chromosomes and from their smallness, that it would be easy to recognize the sex chromosomes even in the heterozygous sex, which, according to Vandel's theory of sex determination (see later), is the female.

Radu also cites Nichols as finding in 1902 no heterochromosomes in the male woodlouse *Oniscus asellus*, in which the chromosome number is $n=16$ and $2n=32$, nor did Vandel (1934) observe heterochromosomes in the male of the woodlouse *Trichoniscus elisabethae* in which $n=8$ and $2n=16$, and which also has fairly large chromosomes.

Since it is of considerable importance to know which sex is heterogametic, it would be very valuable to examine oogenesis in either *Trichoniscus* or possibly in *Asellus*, in which, according to Vandel (1938a), $n=8$. The whole of Vandel's theory (see later) to account for broods which consist entirely of females or entirely of males depends upon the female being the heterozygous sex, and the cytoplasm controlling the segregation of the sex chromosomes into the eggs and polar bodies.

4. BREEDING AND FEEDING TECHNIQUES

Two methods of keeping *Armadillidium* have been found useful, (a) in Petri dishes and (b) in jars. Adult animals can be kept quite satisfactorily in Petri dishes of 10 cm. diameter and 1.5 cm. depth. Damp filter papers are placed upon the bottom of the dish, and food consisting of dead leaves and twigs fills up most of the dish. About ten adults can be kept in such dishes, but the most convenient number is about four, e.g. at breeding time one male and three females. The dishes must be kept moist and should be examined every week or every two weeks in summer.

It has been found useful to place each Petri dish in a 50 cigarettes box and three such units in a larger cardboard box. This helps to keep the dishes damp.

The animals can also be kept quite satisfactorily in jars. Jars of the 1 lb. jam-jar size with a screw metal top are very convenient. About 1 in. depth of cotton-wool is placed on the bottom of the jar, compressed and well wetted. The rest of the jar is then filled up with dead leaves and twigs. Such jars need only be examined once a month. They should be kept out of direct sunlight, and this is conveniently done by storing 10-20 of them in strong cardboard or wooden boxes.

In practice it has been found useful to keep the mated males and females in Petri dishes and to examine these dishes once a week in spring and summer. As soon as a female is found to be pregnant, i.e. she has a brood pouch, she is placed alone in a jar. Also as soon as a brood is seen in a jar, the female is removed and placed in another jar or dish.

It is important not to handle the animals when they are moulting, for if so they often die. Like other woodlice, *Armadillidium* moults its exoskeleton in two halves, the hind end first and the front part a few days later.

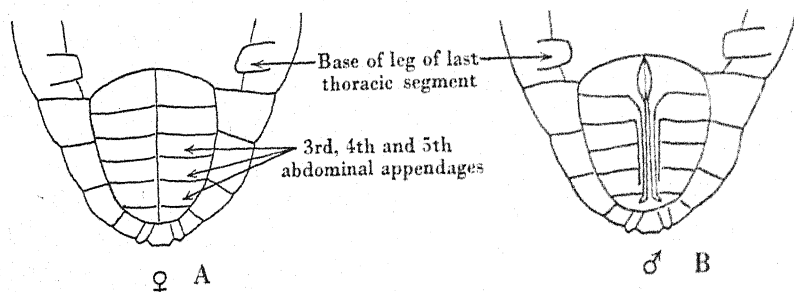
The best food appears to be dead leaves, twigs, and fruits obtained from the ground in beech woods. Care has to be taken that no woodlice are present in this food, but this is not difficult. The animals also eat slices of potato and the cotyledons of pea seeds. Leaf mould from beech woods and slices of potato are recommended. Dead leaves from other than beech woods should not be used. Dead leaves from hawthorn scrub and elm woods have been occasionally tried. On several occasions this has coincided with very small broods being raised from such jars. It is interesting in this connexion to note that *Armadillidium* occurs in beech woods but not in the other two types of wood. On the other hand, the small number of animals raised in some broods appeared to be due to excessive moisture in the jars and not to the wrong types of food.

When disturbed the animals roll up into a ball. This makes handling while scoring easy. The best method is to place the animals in a dry empty Petri dish. They can be very easily picked up when small with a dry paint brush. When adult the fingers or forceps may be used.

Scoring for sex is also quite easy. The male differs from the female in that the first two abdominal appendages bear styles (see Fig. 3). If the animals are placed on dry clean glass, e.g. the bottom of a Petri dish, when they unroll they lie on their dorsal sides with the ventral side uppermost. The styles can then be quite easily seen in the males.

5. GENETICS OF THE VARIETIES

Collinge (1918 *a, b*) has described ten varieties of *A. vulgare*. The commonest varieties, *plumbeus* and *variegatus*, are black or greyish animals. Among the varieties described by Collinge are animals whose colour is "light brownish yellow" (var. *brunneoflavescens*), "deep olive green"



Text-fig. 3. Ventral views of abdomens of male and female. A. Female. No styles. B. Male. Note styles.

(var. *virescens*), and "rich creamy white" (var. *bicolor*). Other varieties are distinguished by colour patterns. Among the few collections I have made, there are types which were not given varietal names by Collinge. Also, as is discussed below, the variety *variegatus* as defined by Collinge includes many distinct phenotypes which are in many cases probably also genotypes. This statement probably also applies to other varieties than *variegatus*.

(a) *Varieties plumbeus* Lereb. and *variegatus* Lereb.

These are the commonest types found. The descriptions given by Collinge are: *plumbeus*—"uniform leaden grey, with the posterior border of the segments whitish"; and *variegatus*—"grey-black with sulphur yellow spots irregularly distributed and variable in number. May be arranged in two longitudinal series or have the ground colour lighter and marbled with grey and yellow."

Very nearly every male animal found in collections around Cambridge is var. *plumbeus* and most of the females are var. *variegatus*. The *variegatus* females, however, include many quite distinct phenotypes; the ground colour varies from the leaden grey found in the males to greys and brownish blacks and even browns, while the markings may be either yellow or white.

As previously stated, the young animals are completely white when born and only gradually become fully pigmented. At one stage in their life young males would be classified as var. *variegatus* but they gradually

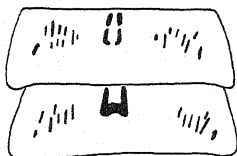
lose the *variegatus* series of markings. This loss may be very slow in some cases, and it was such a slow loss which led to the statement in Howard (1938) that an "exceptional" male in a thelygenic brood was of the *variegatus* type. The females of some broods also lose the *variegatus* markings as they grow older and so become variety *plumbeus*. The rate of loss of these markings appears to differ in different broods.

Vandel (1939 *b*) has summarized the previous knowledge about varieties *plumbeus* and *variegatus*, with observations of his own. His remarks agree entirely with mine. Thus Dolfus had noticed the sexual dimorphism and J. Carl the change with age of animal.

In my nomenclature animals of variety *plumbeus* are called "black, type A" and one type of *variegatus* is "black, type B". These two types are illustrated in A and B of Pl. IV. Females which would be included in var. *variegatus*, as defined by Collinge, may also be "grey, type B", or "brownish black, type B" or "marbled". As is shown in B and C of Pl. IV, type B is characterized by a regular series of white or yellow markings in each thoracic segment. A diagram of type B markings is also shown in Text-fig. 4. The markings consist of blotches in the median line and a series of lines on either side. The black pigment is not found in the exoskeleton which is shed at moults, but in a layer immediately beneath it. The white or yellow markings are due to an absence of this black pigment.

(b) *Red varieties*

A "red" variety of *Armadillidium* is rather common around Cambridge (see later for analysis of populations). It is probably var. *rufobrunneus* Collinge. The description of this variety given by Collinge is "ground colour uniform reddish brown, with the pleural plates lighter in



Text-fig. 4. Diagram of type B markings. 4th and 5th thoracic segments of a female.

colour" (cf. Pl. IV, C). Var. *brunneoflavescens* Collinge may also occur near Cambridge. The description of this variety is "whole of dorsum light brownish yellow". One animal of a lighter colour than the red ones examined genetically has been obtained. Another animal of a much darker red than var. *rufobrunneus* has also been obtained. In the red variety the eyes and mandibles are still black and not red.

As was reported earlier (Howard, 1938), red is dominant to the commoner black or greyish colour. The full genetical results are given in Tables II and III. It will be seen that two homozygous red females have been obtained. These animals cannot be distinguished by their phenotypes from the heterozygotes. As was to be expected, all the wild animals were heterozygotes. Red is neither sex-linked nor sex-limited.

TABLE II

Genetical results for red v. black

(a) 3 : 1 ratio		
Brood 1	♀ A (red) × ♂ B (red)	27 red : 13 black
(b) 1 : 1 ratio from A		
Brood 5	♀ A (red) × ♂ S (black)	5 red : 5 black
(c) 1 : 1 ratios from B		
Brood 6	♀ D (black) × ♂ B (red)	5 red : 11 black
Brood 7	♀ C (black) × ♂ B (red)	4 red : 8 black
Brood 8	♀ AB (black) × ♂ B (red)	16 red : 16 black
(d) Progeny of brood 1 (brood 1 animals BA-BO)*		
(i)	Black ♂ BA × ♀ AF (black)	58 black : 0 red
(ii)	Black ♀ BG × ♂ AK (black)	36 black : 0 red
(iii)	Red ♀ BB × ♂ G (black)	16 red : (1 blackish ?)
	Red ♀ BK × ♂ AN (black)	45 red : 0 black
(iv)	Red ♀ BC × ♂ G (black)	24 red : 29 black
	Red ♀ BD × ♂ G (black)	23 red : 27 black
	Red ♀ BN × ♂ SA (black)	27 red : 27 black
	Red ♀ BO × ♂ SA (black)	18 red : 20 black
(e) Progeny of brood 7 (♀ CE)		
Black ♀ CE × red male of same brood		28 red : 15 black
(f) Other broods		
♀ Y (black) × unknown male (red ?)		33 red : 28 black
♀ GA (black) × ♂ JA (red)		9 red : 17 black
♀ JD (red) × unknown male (black ?)		84 red : 68 black
♀ JB (red) × ♂ SG (black)		132 red : 135 black

* N.B. Of the six red females, four are heterozygous and two homozygous.

Animals of var. *rufobrunneus*, i.e. in my terminology red as opposed to black, may be either type A if they are males or type B if they are females. If we used Collinge's descriptions of the varieties, only red males would be var. *rufobrunneus*, and we should have to make another varietal name for red females.

(c) *Type C*

This variety does not appear to have been described by Collinge. It differs from types A and B in that the posterior margins and outer edges of the thoracic segments are brownish red and that most of the abdominal segments (the outer plates of the telson excepted) are also largely

brownish red to white. The thoracic segments may also have a blob of reddish brown in the median line. The outer plates of the telson are black. Animals of type C change with age in respect of the reddish brown areas,

TABLE III

Summary of 1 : 1 ratios for red v. black

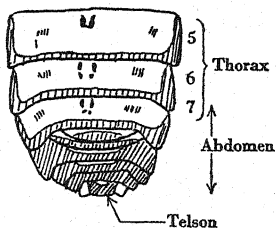
Parents of broods	Red	: Black	Total animals
(a) Red ♀ A	5	5	10
(b) Red ♂ B	25	35	60
(c) Red females of brood 1	92	103	195
(d) Red male of brood 7	28	15	43
(e) Other red males	42	45	87
(f) Other red females	216	203	419
Total	408	406	814

Analysed for sex also

Analysis for sex was often later than the first scoring for red v. black.

Brood from	Females		Males	
	Red	Black	Red	Black
♂ C (black)	0	5	3	0
♂ AB (black)	6	3	9	13
♂ BC (red)	7	10	17	19
♂ BD (red)	15	19	8	8
♂ BN (red)	6	8	20	18
♂ BO (red)	8	6	10	12
♀ CE (black)	25	14	3	1
Totals	67	65	70	71

but they are always easily recognizable. In young animals, the whole of the abdominal segments (outer plates of the telson excepted) may be almost white. Text-fig. 5 shows diagrammatically the distribution of the



Text-fig. 5. Type C. Two-year-old female. 5th, 6th, 7th thoracic segments and the abdominal segments. White areas in diagram, black in the animal; black markings in diagram, see type B; shaded areas in diagram, reddish brown or white in the animal.

pigments in type C, and this variety is also shown in E of Pl. IV. In distribution of pigment the variety corresponds to a certain extent with that described as *marginalatus* Collinge—"ground colour of dorsum dark grey, with lighter lateral patches and broken median line; pleural plates light yellow". A variety similar to type C but with yellow pigment instead of reddish brown occurs near Cambridge.

It is found in examining wild populations that type C animals are always females. Also, if broods from type C females \times type A males are scored when the animals are at least six months old, all the males are type A, and of the females one-half are type B and the other half type C. But it has also been found that type A males can carry the factor for type C. In adult animals, therefore, type C appears to be sex-limited and dominant to B.

TABLE IV
Genetics of type C

Brood 1. ♀ A (type B) \times ♂ B (type A)	♂ BA and 36 ♀♀ (incl. BK), all type B
Brood 2. ♀ C (type C) \times unknown ♂	4 ♂♂, type A, AK, AL, AM, AN 4 ♀♀, type B, AD, AF, AH, AJ 4 ♀♀, type C, AB, AC, AE, AG ♂ CA, ♀♀ CB, CC, CD. All type B
Brood 4. ♀ E (type B) \times unknown ♂	3 ♂♂, type A
Brood 7. ♀ C (type C) \times ♂ B (type A)	1 ♀, type B 4 ♀♀, type C (incl. ♀ CE)
♀ AB (type C) \times ♂ B (type A)	22 ♂♂; 4 ♀♀, type C; 5 ♀♀, type B
♀ AF (type B) \times a brood 2 ♂♂ (type A, carrying type C?)	1 ♂; 2 ♀♀, type B; 1 ♀, type C
♀ AF (type B) \times ♂ BA (type B)	43 ♀♀ : 15 ♂♂, all type B
♀ AJ (type B) \times ♂ AM (type A, carrying type C?)	2 type B; 6 type C
♀ AB (type C) \times ♂ AM (type A, carrying type C?)	26 type C; 16 type B (hard to score, mostly males)
♀ CB (type B) \times ♂ AL (type A, carrying type C?)	1 ♂; 20 ♀♀, type B; 17 ♀♀, type C
♀ CC (type B) \times ♂ AL (type A, carrying type C?)	0 ♂♂; 12 ♀♀, type B; 13 ♀♀, type C
♀ CD (type B) \times ♂ AL (type A, carrying type C?)	All ♂♂, 30 type B, 34 type C
♀ BK (type B) \times ♂ AN (type A, not carrying type C)	All ♂♂ and all type B
♀ U (type B) \times ♂ AN (type A, not carrying type C)	All ♀♀ and all type B
♀ CE (type C) \times ♂ of the same brood	4 ♂♂, 3 type B : 1 type C 39 ♀♀, 19 type B : 20 type C

However, it has been found that, if broods are scored early, young males may be type C or type C-ish. As these type C males grow older they become type A (cf. type B males becoming type A). Although the genetical evidence for the fact that type A males can carry the factor for type C is good enough, it still needs to be confirmed that young males classified as type C-ish do really carry the type C factor.

The genetical data for type C variety are given in Table IV. It can be seen that type C is dominant to type B, and that in adult animals it is sex-limited to the female sex. It must, however, be pointed out that neither males nor females homozygous for the type C factor have yet been obtained.

Type C red females have also probably been obtained. These are rather difficult to recognize because the type C markings of a black animal are also red. It did, however, appear possible to recognize type C red females by a light blotch in the median line and by the two outer plates of the telson appearing darker than the rest of the abdomen in young animals. Such females have not yet produced broods.

(d) *Type D*

In collections around Cambridge females only of this variety have been found. The genetical data are very meagre, but young males do appear to be type D-ish and later to become type A. Also in females type D is dominant to the commoner type B (see Table V). The factor for type D therefore seems to be similar to that for type C, in that its expression in adult animals is limited to the female sex.

TABLE V

Genetics of type D

♀ DB (type D) × ♂ SC (type A)	♂♂, types A + B 7 : type D-ish 11 ♀♀, type B 25 : type D 25
♀ DA (type D) × ♂ SB (type A)	0 ♂♂; 17 ♀♀ type D; 13 ♀♀ type B
♀ DE (type D) × ♂ SD (type A)	♂♂, type B 9 : type D-ish 8 ♀♀, type B 19 : type D 16

Type D, as recorded in the analysis of populations (see later), contains almost certainly two or more distinct types. One of these types is illustrated in F of Pl. IV. Another type has the ground colour of the thorax a much lighter colour than that illustrated. This latter type approaches the variety illustrated in D of Pl. IV. Type D does not appear to have been described by Collinge. Type D differs from type B in that the general colour of the whole of the body, except for the head and outer plates of the telson, is brownish yellow to yellow, in the edges of the thoracic segments being light yellow to white, and in the abdomen also being a lighter colour than the thorax. Animals homozygous for the type D factor have not yet been obtained.

(e) *Var. cooperi Collinge*

The description of this variety given by Collinge is: "Cephalon blackish brown, finely speckled with yellow. Pleural plates of the mesosome pale yellowish white, also the posterior border of the segments. On each side of the median line is a somewhat oval-shaped purplish brown patch with yellowish wavy stripes, the two patches generally meeting anteriorly on the first three or four segments. Metasome yellowish

brown." This description gives some idea of the difficulty of describing varieties. Diagrams and coloured plates would seem to be an advantage.

Vandel (1939 *b*) has studied the genetics of forms which are probably var. *cooperi*. He finds this type to be dominant to the commoner varieties *plumbeus* and *variegatus*. The variety is not sex-limited. Vandel also finds that the animals change in colour as they grow older from yellow ("beau jaune citron") to brown ("bistre"). Prof. Vandel kindly gave me some animals of this variety and one of them is figured in D of Pl. IV. While some of them were different from any animals I have found near Cambridge (particularly the yellow ones like that shown in the plate), others were brownish and very similar to types which I should place in type D. I have not yet been able to study the change with age. It was, however, possible to confirm that the variety is not sex-limited since there were var. *cooperi* males in the animals given me. This suggests that perhaps in some collections in this country type D might not be found to be sex-limited.

(f) *Other types*

I have at various times found other varieties of females from those described above, but have not bred from them. Also, as was pointed out earlier, var. *variegatus* as defined by Collinge includes several distinct types. One of these types I have called marbled and this type appears to be dominant in females to the more usual type B. Marbled females are distinguishable from type B females by the ground colour of the body being black flecked with brown as opposed to pure black.

Also, as was pointed out earlier, type B females may be black, greyish, or brownish. The genetics of these colours would not be easy since in many cases they appear to grade from one type to another. Brown appears to be dominant to black and is also sex-limited to females.

(g) *Summary and discussion*

Although the genetical results are not yet numerous, and collections of animals have only been obtained from a few localities around Cambridge, two points stand out. First the rarer types are dominant to the common types and secondly some of the varieties are sex-limited.

The occurrence of the sex-limited varieties (types C and D) is also very interesting. Vandel (1938 *b*) has suggested that the female is the heterozygous sex in woodlice. If this is so, then *Armadillidium* would resemble the woodlice in having a part of its polymorphism confined to

the heterozygous sex, which in the case of the Lepidoptera is also the female (Fryer, 1913; review by Ford, 1937).

The *Armadillidium* case especially resembles that found in *Papilio polytes* by Fryer (1913). In *P. polytes* the mimetic types are dominant to the normal type and are also sex-limited to the females, although the males can carry the factors for the mimetic types. Fryer's explanation of these results (see especially footnote on p. 237 of Fryer's paper) can be applied to the *Armadillidium* case as is shown below:

$X^P X^P A^C A^C$	male, type B when young, type A when mature,
$X^P X^P A^C A^C$	male, type C when young, type A when mature,
$X^P Y^P A^C A^C$	female, type B when young, type B when mature,
$X^P Y^P A^C A^C$	female, type C when young, type C when mature,

(**X** and **Y**=sex chromosomes; **A**=an autosome), i.e. in adults homozygous for **P** (males), **PP** inhibits the action of the gene **C**, but in females (constitution **Pp**) the gene **C** is not inhibited. Or perhaps, as suggested to me by Prof. Punnett, in the *Armadillidium* case it would be better to say that **P** is a gene for producing the black pigment and it is only **Pp** which can be modified by the action of the gene **C**.

As an alternative explanation, Cockayne (1932) has suggested that females only would show the sex-limited characters if an autosomal gene could operate only in the presence of another gene carried by the Y-chromosome. But as Ford (1937) points out, we have also to explain why the auxiliary factor in the Y should more often be activating than inhibiting. The present cases in *Armadillidium*, if young males really do show type C or type D patterns, adds a further complication to this story. A factor in the Y-chromosome appears to be stopping a process which in the absence of a Y can change type C or D into type A.

A third alternative is that suggested by Ford (1937, p. 491). "It is usually held that such sex-controlled inheritance is due to the operation of autosomal genes whose action is inhibited in the internal environment provided by one of the sexes, usually the male." This type of explanation does not appear to fit the *Armadillidium* cases because young males do show type C and type D markings.

It must be also pointed out that var. *cooperi* animals of Prof. Vandel (both males and females found) resemble type D animals (only females found). It may therefore be that only in certain races of *Armadillidium* is sex-limited inheritance found. Further work is required on this point.

6. SEX RATIOS

The first brood scored for sex consisted of 1 male : 36 females, although it showed an apparently normal red : black segregation (Howard, 1938). Many other broods have since been found to contain a deficiency of males or females from the expected 50 % males : 50 % females. Vandel (1939 *a*) has also reported cases of such monogenic broods in *Armadillidium vulgare*.

(a) Terms used

It is proposed to use the following terms in describing the different types of broods and females. They are taken from Vandel's (1938 *b*) paper on monogeny in the woodlouse *Trichoniscus provisorius*. This paper of Vandel is a large one, and much of what follows in the present paper on the theory to account for the monogenic broods is also taken from Vandel (1938 *b*). The terms used are (and see also Table VI):

(1) *Amphogenic* females produce amphogenic broods which contain approximately equal numbers of males and females.

TABLE VI

Examples of the different types of sex ratios found in broods

	Males	Females	Total animals 2	<i>d</i>	<i>σ</i>
(1) From amphogenic females					
Brood 24 from female EA	46	47	46.5	0.5	4.77
Brood 20 from female H	23	29	26	3	3.61
(2) From monogenic females					
(a) Strong arrhenogenics					
Brood 35 from female CD	64	0	32	32	4.00
Brood 33 from female BK	45	0	22.5	22.5	3.35
(b) Strong thelygenics					
Brood 48 from female U	0	56	28	28	3.72
Brood 29 from female CB	0	37	18.5	18.5	3.04
(c) Weak arrhenogenics					
Brood 25 from female AB	34	8	21	13	3.24
Brood 27 from female BC	36	17	26.5	9.5	3.64
(d) Weak thelygenics					
Brood 43 from female CE	4	39	21.5	17.5	3.28
Brood 49 from female AF	15	43	29	14	3.81

(2) *Monogenic* females produce monogenic broods which contain animals of one sex only or very nearly so. They are of two types:

- (a) *Arrhenogenics*—producing mostly male offspring.
- (b) *Thelygenics*—producing mostly female offspring.

It will be noticed that the terms apply both to the broods and to the

females producing that type of brood. Vandel also uses other terms, but these are mostly not needed for the present discussion.

It can be seen in Table VI that there appear to be degrees of "arrhenogeny" and "thelygeny". Vandel (1938 *b*) distinguishes between *perfect* arrhenogenics which produce broods consisting of males only and *imperfect* arrhenogenics which produce broods containing a small percentage of females and a high percentage of males. It would seem from the data given in Table VI that we might call female CD strongly arrhenogenic and females AB and BC weakly arrhenogenic. It would also appear that it is in cases such as these that the terms arrhenogenic and thelygenic are more convenient than the simpler ones, "male-producing" and "female-producing", as applied to females. The terms arrhenogenic and thelygenic are also very useful when referring to broods. If these terms were not used, one would have to talk about "completely male" broods, broods "with a preponderance of males", etc.

(*b*) *Summary of Vandel's work on Trichoniscus*

My interpretations and explanations of the sex-ratio results in *Armadillidium* are based upon those given by Vandel (1938 *b*) in describing monogeny in the woodlouse *Trichoniscus*. A summary of Vandel's results and his theory to account for monogeny will therefore be given before discussing the *Armadillidium* results.

Vandel found that monogenic broods are not smaller in size than amphogenic ones. This means that monogenic broods are not due to the elimination in the developing eggs or in young animals of either of the sexes. He also claims to have shown that the male has no effect on the type of brood produced. His evidence for this statement is that broods fathered by both "mâles ordinaires" (i.e. mâles from amphogenic broods) and by "mâles exclusifs" (i.e. males from arrhenogenic broods) can be either arrhenogenic or thelygenic (see Table VI of Vandel, 1938 *b*), and he also showed that in several cases sister females mated with different types of males produced the same type of brood. Thus in three cases all the females of a brood produced arrhenogenic broods and in one case all the females of a brood produced thelygenic broods irrespective of the type of male with which the different females of the same brood were mated (see also Howard, 1939). Also Vandel has never seen any intersexes and so it is unlikely that for example part of the males in an arrhenogenic brood are transformed females (cf. *Lymantria*).

Vandel's theory to account for amphogenic and monogenic broods is simple. In woodlice he suggests that the female is heterozygous sex

(chromosomes XY) and the male the homozygous sex (chromosomes XX). Amphogenic females produce 50 % of eggs containing the X -chromosome and 50 % of eggs containing the Y -chromosome. In monogenic females, on the other hand, there is a selective segregation of the X - and Y -chromosomes. For example, in arrhenogenic females the Y -chromosome always goes into the first polar body and thus every egg contains an X -chromosome. All the offspring are therefore of the constitution XX and therefore all are males.

Vandel also considers that the segregation of the sex chromosomes is controlled by the cytoplasm of the female, and he states that "the monogeny is a property which follows the maternal or cytoplasmic inheritance". Thus thelygenic females produce thelygenic daughters, amphogenic females amphogenic daughters, and the exceptional females in arrhenogenic broods are themselves arrhenogenics. This, however, is only partially true. An inspection of Table XIII of Vandel (1938 *b*), for example, shows that the daughters of perfect thelygenics may even be perfect arrhenogenics, and Vandel also states "the arrhenogenic type and the thelygenic type seem to correspond to two states of balance of the cytoplasm. The type may be transmitted without modification from one generation to the next, or on the contrary it reverts partially or totally."

(c) *General considerations of monogeny in Armadillidium*

One possible explanation of the unisexual broods might be that they were due to parthenogenesis. It has also been pointed out that parthenogenesis does not occur (see p. 85) and the genetical results given in Table VII also show that neither arrhenogenic nor thelygenic broods are due to

TABLE VII

Segregations in arrhenogenic and thelygenic broods

1. Female CE (black) \times red male, 4 males : 39 females.
 Males 1 black : 3 red.
 Females 14 black : 25 red.
2. Female CB (type B) \times male AL (carrying type C), 1 male : 37 females.
 Females 20 type B : 17 type C.
3. Female CC (type B) \times male AL (carrying type C), 0 males : 25 females.
 Females 12 type B : 13 type C.
4. Female CD (type B) \times male AL (carrying type C), 64 males : 0 females.
 Males 30 type B : 34 type C.

parthenogenesis. The results would be more satisfactory if they did not involve in most of the cases the sex-limited character type C.

In Toulouse the number of broods produced by a female per year is three. Vandel (1939 *a*) has found that in successive broods from the same female the percentage of males tends to increase from first brood to third.

Table VIII shows some of Vandel's results. Both these females are described as amphogenics by Vandel, although all six broods differ signi-

TABLE VIII

Changes of sex ratios of successive broods from same female. Data from Vandel (1939 a)

	Males	Females	% males	Total animals 2	d	σ
(1) Female A 2						
1st brood	29	89	24.5	59	30	5.4
2nd brood	111	31	78	71	40	6.0
3rd brood	123	11	91.7	67	56	5.8
(2) Female A 15						
1st brood	44	68	39	56	12	5.3
2nd brood	47	26	64.3	36.5	10.5	4.3
3rd brood	66	25	72.5	45.5	20.5	4.8

ficantly from 50 % males : 50 % females. These results also suggest that great care is necessary in interpreting the results of sex ratios in inheritance studies. Vandel (1938 b) also found that in *Trichoniscus* the percentage of males in broods changed with time of year. The number of females in broods decreased markedly in October, November and December.

There are not yet many data to show how sex ratios of broods from the same female vary from year to year. Data for two females are given in Table IX and the results suggest that in some cases the female does not change in type of brood produced.

TABLE IX

*Changes in sex ratios in successive broods from same females.
Intervals between broods one year*

(1) Female AB		
1938	22 males : 9 females	% males = 71
1939	34 males : 8 females	% males = 81
(2) Female U		
1938	0 males : 12 females	
1939	0 males : 56 females	

There are several results suggesting that in *Armadillidium* the male has no effect on the type of brood produced (cf. Table X). Thus a male may be parent to an arrhenogenic brood from one female and to a thelygenic brood from another female, or to an amphogenic from one female and a thelygenic from another. Hence the male would appear to have no effect on the type of brood produced. This is the best evidence so far

found to support Vandel's theory of accounting for monogenic broods, see also Howard (1939).

TABLE X

Sex ratios in broods by same male from different females

(1) Male B					
1937	× female A	1 male : 36 females			
1938	× female AB	22 males : 9 females			
(2) Male AM					
1939	× female AB	34 males : 8 females			
1939	× female AJ	6 males : 50 females			
			Total animals		
(3) Male G			2	d	σ
1939	× female BC	36 males : 17 females	26.5	9.5	3.6
1939	× female BD	16 males : 34 females	25	9	3.5
(4) Male AL					
1939	× female CB	1 male : 37 females			
1939	× female CC	0 males : 25 females			
1939	× female CD	64 males : 0 females			
(5) Male AN					
1939	× female BK	45 males : 0 females			
1939	× female U	0 males : 56 females			
(6) Male SB					
1939	× female DA	0 males : 30 females			
1939	× female EA	46 males : 47 females			

(d) Frequency of occurrence of amphogenic and monogenic broods

Table XI shows the results obtained so far. It will be seen that very few females produce broods which contain approximately equal numbers of males and females. This immediately raises the question as to what is a true amphogenic brood and as to how important environment is in affecting the sex ratio (see also Table VIII). For example, broods of 20 females : 40 males and 40 females : 20 males (both of which differ from 30 females : 30 males by more than twice the standard deviation) may both be from amphogenic females and the excess of one sex may be due to environmental factors. We have also to ask whether the sex ratio in true amphogenic broods is 100 females : 100 males. Thus in animals in which the male is the heterozygous sex, the sex ratio very often deviates from strict equality, e.g. according to Whitney (1939) in German shepherds (dogs) the sex ratio in litters is 143.1 males : 100 females in the colder months and 116.1 males : 100 females in the warmer months. Crew (1937) and Mayr (1939) have also discussed sex ratios.

Crew (1937) gives the following figures. For England and Wales in new-born babes the sex ratio is 105.6 males : 100 females, and for deaths in the 7th-9th months of uterine life 110 males : 100 females. For albino

rats the figures are 106 males : 100 females at birth and of still-born young 129 males : 100 females. These figures show that in man and in rats (male is the heterozygous sex in both cases) the primary sex ratio (i.e. proportion of sexes at fertilization) is not 100 : 100 but about 110 males : 100 females or even further from equality.

TABLE XI

Summary of sex ratios in broods

Brood no.	Female parent	Constitution of brood		Total animals	d	σ	Type of brood
		Males	Females				
		(1) Females from collections					
1	A	1	36	18.5	17.5	3	T
20	H	23	29	26	3	3.6	A
22	FA	0	49	24.5	24.5	3.5	T
23	DB	17	50	33.5	16.5	4.1	(T)
24	EA	46	47	46.5	0.5	4.8	A
38	KA	1	24	12.5	11.5	2.5	T
42	JC	22	14	18	4	3	A
47	DA	0	30	15	15	2.7	T
54	DE	17	35	26	9	3.6	(T)
(17+48)	U	0	68	34	34	4.1	T

I.e. there are 3 amphogenics, 5 strong thylogenics, and 2 weak thylogenics.

(2) Daughters of female A (brood 1—1 male : 36 females)

27	BC	36	17	26.5	9.5	3.6	(M)
28	BD	16	34	25	9	3.5	(F)
33	BK	45	0	22.5	22.5	3.4	M
34	BN	38	14	26	12	3.6	(M)
39	BO	22	14	18	4	3	A

(3) Daughters of female C (broods 2+7—7 males : 13 females)

8+25	AB	56	17	36.5	19.5	4.3	(M)
26	AJ	6	50	28	22	3.7	T
49	AF	15	43	29	14	3.8	(T)
43	CE	4	39	21.5	17.5	3.3	T

(4) Daughters of female E (brood 4—1 male : 4 females)

29	CB	1	37	19	18	3.1	T
30	CC	0	25	12.5	12.5	2.5	T
35	CD	64	0	32	32	4	M

A=amphogenic brood; T=thylogenic brood; M=arrhenogenic brood.
(T)=weakly thylogenic; and (M)=weakly arrhenogenic.

Data for birds, in which the female is the heterozygous sex, are also available. Crew (1937) states that in fowls the percentage of males at hatching is 49.17 ± 0.11 and in dead embryos 47.85 % are males. Mayr (1939) quotes Cole and Kirkpatrick to have found that in the domestic pigeon the secondary sex ratio is 105 females : 100 males. McIlkenny, also quoted by Mayr (1939), found in *Cassidix texanus* that the primary sex ratio for 37 nests was 34 males : 77 females (30.3 % males) and the secondary sex ratio for 89 nests was 70 males : 135 females (34.1 % males).

While, except for *C. texanus* where the numbers are small, the sex ratio does not deviate widely from 100 : 100, the figures do suggest that in true amphogenic broods the sex ratio might be 120 females : 100 males. This might then mean that a brood of 40 females : 20 males was a true amphogenic one but a brood of 20 females : 40 males was a weakly arrhenogenic one. On the other hand the possible effects of environment or change with age of female (see Table VIII) would seem to be of more importance.

It will be seen from Table XI that of the ten females obtained from collections of wild animals 3 are true amphogenics (females H, EA, and JC), 5 are true thelygenics (females A, FA, KA, DA, and U) and 2 are imperfect thelygenics (females DB and DE). There were thus no arrhenogenics but several were found in the progeny of females A, C and E. Vandel (1939 *a*) found in *Armadillidium* that of 15 females 7 were "amphogenics" (including, however, females such as A 2 and A 15 of Table VIII of this paper), 4 were arrhenogenics, and 4 were thelygenics.

(e) *The inheritance of monogeny*

According to Vandel (1938 *b*) in *Trochoniscus*, "the monogeny is a property which follows the type of the maternal or cytoplasmic inheritance". Thus amphogenic females produce amphogenic daughters and monogenic females produce monogenic daughters. Also according to Vandel "the arrhenogenic type and the thelygenic type seem to correspond to two states of balance of the cytoplasm. The type may be transmitted without modification from one generation to the next, or on the contrary it reverts partially or totally". Thus in *Trichoniscus* (Table VII of Vandel, 1938 *b*), of 38 exceptional females (i.e. the occasional females in arrhenogenic broods) 15 were perfect arrhenogenics like their mothers and 12 were imperfect arrhenogenics. No perfect thelygenics occurred. Also of 52 females from perfect thelygenics broods (Table VIII of Vandel, 1938 *b*), only 8 were perfect thelygenics and no less than 17 were perfect arrhenogenics (i.e. these latter animals inherited monogeny from their mothers but it was the opposite type of monogeny). The average size of broods in *Trichoniscus provisorius* is only 7 and thus the classification of broods may often be doubtful, particularly that of the imperfect (= weak) monogenics.

The data so far obtained for *Armadillidium* are given in Table XI. They are not very extensive, but they do show two things. First the daughters of a thelygenic female (brood 1) include a strong arrhenogenic

and several weak arrhenogenics. Secondly, it is possible to find in the same brood both strong arrhenogenics and thelygenics (brood 4).

One possible way of explaining these data is that, though the male has no effect on the sex ratio in the brood itself, he does have an effect on the type of daughters produced. A possible scheme is outlined in Table XII. It is also obvious that all such schemes must include a means by which arrhenogenic daughters are produced by non-arrhenogenic females. If there were no such means, then arrhenogenic females would cease to exist since they do not produce daughters themselves or only a very small number of daughters. An alternative to any scheme similar to that given in Table XII is that there is a high mutation rate producing arrhenogenic females.

TABLE XII

Possible genetical relationships of amphogenics and monogenics

AAbb	} Strong arrhenogenics	AaBb	Weak arrhenogenic?
Aabb		AABB	} Amphogenics?
aabb		AaBb	
		AaBB	Weak thelygenic?
aaBb	} Strong thelygenics		
aaBB			

Thus, for example, female **aaBb** (strong thelygenic) × male **Aabb**.
 Daughters: 1 **Aabb** Strong arrhenogenic
 1 **AaBb** } Both amphogenics
 1 **aabb** }
 1 **aaBb** Strong thelygenic

In the scheme suggested in Table XII the control of the movement of the sex chromosomes would still be in the cytoplasm, but the constitution of the cytoplasm would be determined by the chromosomes (cf. sinistrality and dextrality in the snail *Limnaea peregra* (Diver *et al.* 1925)). An obvious complication of the scheme is that there may exist A's and B's of different strengths so that, for example, **A** (strong) **aB** (weak) **b** may be weakly arrhenogenic and not amphogenic.

The best method of attacking the problem of amphogeny and monogeny would appear to be to inbreed and obtain a strain of amphogenics which breed true for a constant sex ratio of 50 : 50. Females of this strain could then be crossed with males from arrhenogenic and thelygenic broods to see what types of daughters are produced. In spite of Vandell's results (see Table VIII of this paper), *Armadillidium* would appear to be a more suitable animal for such researches than *Trichoniscus* because of the larger size of its broods.

(f) Discussion on monogeny

One would assume that in animals with an XY-chromosome mechanism the primitive condition is that the sex ratio should be approximately 100 males : 100 females. Monogeny would then be a specialized condition and it is therefore interesting to see what its effects are. The most obvious effect is that it restricts inbreeding. The daughters of a thelygenic brood cannot mate with their brothers because no such animals exist. A second possible effect is that the tertiary sex ratio (i.e. the ratio in adults) could easily be 1 male : 2 females, for example, if there was 50 % of amphogenic females and 50 % of thelygenic females. It is interesting in this connexion to note that one male can fertilize at least three females a year (e.g. male AL of Table VII) and that the tertiary sex ratio in many populations (see later) does show many more females than males. Monogeny would also appear to be a danger in certain circumstances. If only one pregnant female reaches a suitable habitat and if she is either a strong arrhenogenic or thelygenic, then no further broods are possible from her offspring.

Peculiar sex ratios are also found in flies of the genus *Sciara* (review by Metz, 1938). In *Sciara coprophila* there are found two types of females, male-producing females (XX) and female-producing females (XX'). The sex-determining mechanism takes place after fertilization and consists of a different type of elimination of sex chromosomes in eggs of male-producing females to that found in eggs from female-producers. Female-producing females (XX') also produce equal numbers of female-producing females (XX') and of male-producing females (XX). Exceptional amphogenic (digenic) females were found to have sex chromosomes of the constitution XXX (Reynolds, 1938). However, in other species of *Sciara* conditions are not so simple. "Some species regularly give unisexual families, others regularly give bisexual families, and still others possess interfertile strains of both types." Also "in species regularly giving bisexual progenies, such as *S. pauciseta*, the sex ratio varies through a wide range on both sides of 1 : 1". These facts have not yet been explained, but in discussing the nature of the problems, Metz (1938) makes several suggestions: "On this basis, however, it would be expected that all females would be alike (X"X) and that males and females would be produced in equal numbers. This would apparently require the assumption that eggs receiving X" develop into females and those receiving X develop into males. Thus the factors influencing chromosome elimination would be effective after, rather than before, maturation of the egg. To account

for the variable sex ratio on this basis alone it would be necessary to assume that segregation of X'' and X in the egg is not random, that in some females one and in some the other tend to be retained in the egg rather than go off in the polar body." Also Metz suggests: "This may permit crossing-over of influential genes between X'' and X and give chromosomes of different strengths which in turn might account for the variable sex ratios."

The problems in *Armadillidium* and *Sciara* appear to be similar in several respects. The *Sciara* results also show how necessary a cytological investigation is in such problems. It might be that in woodlice also sex determination was by elimination of sex chromosomes in the soma of developing eggs such as is found in *S. coprophila*. Another way in which the segregation of the sex chromosomes might be explained is suggested by the *Drosophila* case described in the next paragraph.

A "sex ratio" gene is known in several species of *Drosophila* (review by Dobzhansky, 1939). Males carrying this gene mated to any type of female produce broods consisting of females only. Such broods are similar in size to the normal amphogenic ones. The gene appears to be carried by the X -chromosome and has no effect in either the heterozygous or homozygous condition in the female. Sturtevant & Dobzhansky (1936) have shown how the gene acts. In males carrying this gene the X - and Y -chromosomes fail to pair at prophase of meiosis. The X -chromosome is also clearly 4-partite instead of 2-partite at first division, and it divides at both first and second divisions. The Y -chromosome shows overcontraction, does not come on to the plate, and is eliminated. All sperms therefore contain an X -chromosome and hence only females are produced.

This *Drosophila* case suggests another way in which thelygenic and arrhenogenic broods may be produced. In arrhenogenic broods the X -chromosome divides twice and the Y does not come on to the plate. In the production of thelygenic broods the Y divides twice and the X does not come on to the plate. Weakly arrhenogenic and thelygenic broods are produced by genes of low penetrance. Again one has to emphasize how important cytological investigations would be in understanding monogeny in woodlice.

7. ANALYSIS OF NATURAL POPULATIONS

A few populations from near Cambridge have been analysed and the results are given in Table XIII. The results are quite accurate for sex, for type A males, for red animals and for type C animals. Type B females,

however, include more than one genotype and phenotype and the same may be so for type D females.

The results show quite clearly that the commonest types of animals are black type A males and black type B females. Red, type C and type D animals (all dominants genetically) are uncommon types.

The results also show that in some populations the number of females is markedly higher than 50 %. Such a marked excess of females in collections of adults is found in other woodlice. Vandel (1925) in his review of reproduction in woodlice gives examples on p. 358; e.g. in *Trichoniscus flavus*, 28 males : 67 females; in *Ligidium hypnorum*, 29 males : 98 females.

TABLE XIII
Analysis of several populations from around Cambridge

Type of animal	Collection						Total	%
	A	B	C	D	E	F		
Males:								
Black, type A	22	15	10	10	21	19	97	25.5
Black, type B + C-ish	0	0	4	1	3	0	8	2.1
Red, type A	0	2	0	0	0	0	2	0.5
Total males	22	17	14	11	24	19	107	28.2
Females:								
Black, type A	4	1	0	4	0	1	10	2.6
Black, type B	65	29	52	16	34	16	212	55.8
Black, type C	8	0	18	1	0	1	28	7.4
Black, type D	4	0	7	2	0	0	13	3.4
Red, type B	0	1	2	2	0	5	10	2.6
Total females	81	31	79	25	34	23	273	71.8
Total animals	103	48	93	36	58	42	380	100.0

Collection A: Beech woods on Gog Magog Hills.

" B: Field at Trumpington.

" C: Beech woods (farther south than A) on Gog Magog Hills.

" D: Trees on roadside at Madingley.

" E: Base of tree at Fen Ditton.

" F: In old gravel pits on University Farm.

8. SUMMARY

1. The woodlouse *Armadillidium vulgare* is a fairly easy animal to breed. It is an easy animal to handle because (a) it rolls up when disturbed and (b) the female carries the eggs about with her in a brood pouch. The animals are mature when one year old and produce only one brood of about 50-150 young per year. The sexes are easily distinguishable.

2. The two commonest types are var. *plumbeus* (all black) males and var. *variegatus* (blackish or greyish with white or yellow markings) females. Other rarer types are found.

3. Two varieties, a red one and var. *cooperi* (yellow), are simple dominants to the commoner black or grey types. Two other uncommon varieties, types C and D, are also dominants but are sex-limited in adult animals to females.

4. Very few broods consist of approximately 50 % males : 50 % females (amphogenics). Many broods are monogenics, either consisting entirely of males (arrhenogenics) or entirely of females (thelygenics). Other broods may be described as weakly arrhenogenic or weakly thelygenic.

5. The type of brood is determined by the female parent. Males mated to different females may produce both arrhenogenic and thelygenic broods. Monogenic broods are interpreted on Vandel's hypotheses that the female is the heterozygous sex and the segregation of the sex chromosomes into eggs and polar bodies is controlled by the cytoplasm.

6. Preliminary observations on the inheritance of monogeny are given.

7. The frequency of the various types in populations from around Cambridge are recorded. There is also a marked excess of females over males in these populations.

REFERENCES

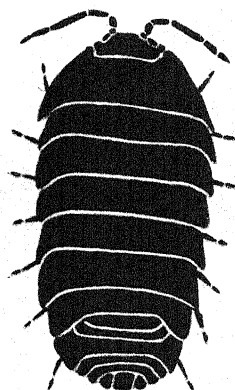
- COCKAYNE, E. A. (1932). "A new explanation of the genetics of sex-limited inheritance in butterflies." *Entomologist*, **65**, 169-76.
- COLLINGE, W. E. (1918 a). "A revised check list of the British terrestrial Isopoda (woodlice), with notes." *J. zool. Res.* **3**, 31-43.
- (1918 b). "Descriptions of some new varieties of British woodlice." *J. zool. Res.* **3**, 101-2.
- CREW, F. A. E. (1937). "The sex ratio." *Amer. Nat.* **71**, 529-59.
- DIVER, C., BOYCOTT, A. E. & GARSTANG, S. L. (1925). "The inheritance of inverse symmetry in *Limnaea peregra*." *J. Genet.* **15**, 113-200.
- DOBZHANSKY, T. (1939). "Experimental studies on genetics of free-living populations of *Drosophila*." *Biol. Rev.* **14**, 339-68.
- FORD, E. B. (1937). "Problems of heredity in the Lepidoptera." *Biol. Rev.* **12**, 461-503.
- FRYER, J. C. F. (1913). "An investigation by pedigree breeding into the polymorphism of *Papilio polytes* Linn." *Philos. Trans. B*, **204**, 227-54.
- HALDANE, J. B. S. (1930). "A note on Fisher's theory of the origin of dominance and on a correlation between dominance and linkage." *Amer. Nat.* **64**, 87-90.
- HOWARD, H. W. (1938). "Genetics of *Armadillidium vulgare* Latr." *Nature. Lond.*, **142**, 1038-9.
- (1939). "Monogenic broods in *Armadillidium vulgare* Latr." *Nature, Lond.*, **144**, 979.

108 *The Genetics of Armadillidium vulgare Latr.*

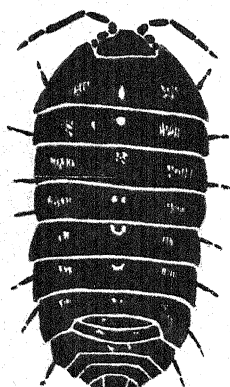
- DE LATTIN, G. (1939). "Untersuchungen über die Farbvariabilität der Isopoden. I. Über genotypische und modifikative Pigmentreduktion." *Zool. Anz.* **125**, 309-24.
- MAYR, E. (1939). "The sex ratio in wild birds." *Amer. Nat.* **73**, 156-79.
- METZ, C. W. (1938). "Chromosome behaviour, inheritance, and sex determination in *Sciara*." *Amer. Nat.* **72**, 485-520.
- NICHOLS, M. L. (1902). "The spermatogenesis of *Oniscus asellus* Linn. with especial reference to the history of the chromatin." *Proc. Amer. phil. Soc.* **41**, 77-112.
- RADU, V. (1930). "Spermatogénèse chez *Armadillidium opacum* (C.-L. Koch)." *C.R. Soc. Biol., Paris*, **105**, 77-8.
- REYNOLDS, J. P. (1938). "Sex determination in a bisexual strain of *Sciara coprophila*." *Genetics*, **23**, 203-20.
- STURTEVANT, A. H. & DOBZHANSKY, T. (1936). "Geographical distribution and cytology of 'sex ratio' in *Drosophila pseudoobscura* and related species." *Genetics*, **21**, 473-90.
- VANDEL, A. (1925). "Recherches sur la sexualité des isopodes. Les conditions naturelles de la reproduction chez les isopodes terrestres." *Bull. biol.* **59**, 317-71.
- (1934). "La parthénogénèse géographique. II. Les mâles triploïdes d'origine parthénogénétique *Trichoniscus elisabethae* de Hérold." *Bull. Biol.* **68**, 419-63.
- (1938 a). "Chromosome number, polyploidy, and sex in the animal kingdom." *Proc. zool. Soc. Lond. A*, **107**, 519-41.
- (1938 b). "Recherches sur la sexualité des isopodes. III. Le déterminisme du sexe et de la monogénie chez *Trichoniscus (Spiloniscus) provisorius* Racovitza." *Bull. Biol.* **72**, 147-86.
- (1939 a). "Sur le mode de répartition des sexes chez l'isopode terrestre, *Armadillidium vulgare* (Latr.)." *C.R. Acad. Sci., Paris*, **208**, 1050-2.
- (1939 b). "Recherches sur la génétique d'*Armadillidium vulgare* (Latr.), dans ses rapports avec la monogénie." *C.R. Acad. Sci., Paris*, **208**, 1351-3.
- WHITNEY, L. F. (1939). "The sex ratio in dogs maintained under similar conditions." *J. Hered.* **30**, 388-89.

EXPLANATION OF PLATE IV

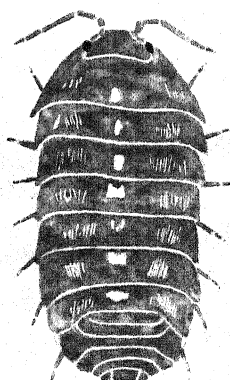
- | | |
|---------------------------|--------------------------------|
| A. Male, black, type A. | B. Female, black, type B. |
| C. Female, red, type B. | D. Male, var. <i>cooperi</i> . |
| E. Female, black, type C. | F. Female, black, type D. |



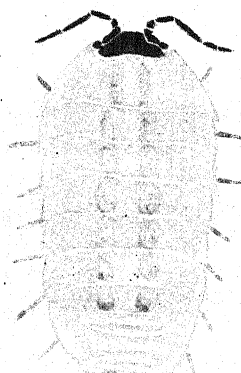
A



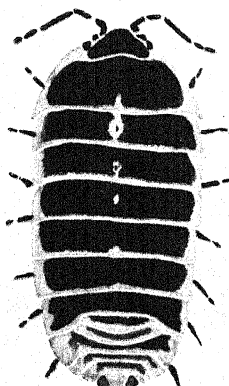
B



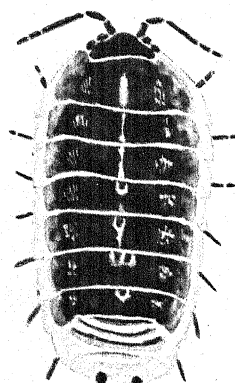
C



D



E



F

REPRODUCTIVE VERSATILITY IN *RUBUS*

I. MORPHOLOGY AND INHERITANCE

By M. B. CRANE

John Innes Horticultural Institution, Merton

CONTENTS

	PAGE
Introduction	109
Reproductive behaviour: (a) Diploid species	110
(b) Polyploids	111
Parthenogenesis	116
Pseudogamy	116
Parthenocarp	116
Summary or reproductive types	116
Summary	117
Acknowledgements	118
References	118

INTRODUCTION

THE results of breeding experiments with *Rubus* at the John Innes Horticultural Institution have been published from time to time (Crane & Darlington, 1927-32; Crane & Lawrence, 1931; Crane, 1936; Lewis, 1939). The object of the present paper is to describe anomalies of breeding behaviour in the genus which have come to light in the course of the investigations.

In *Rubus* the basic chromosome number is 7, and the species we have used range from diploids, $2n=14$, to octoploids, $2n=56$. In the indigenous European species, which we have mainly used, diploids are rare. The raspberry, *R. idaeus*, is mainly diploid; but of thirty British species of blackberries examined during the course of the investigations only one, *R. rusticanus*, the common hedgerow bramble of southern England, is diploid.

In the early years of the experiments assistance in the breeding work and chromosome determinations of species and seedlings were made by Dr A. A. Moffett, Dr M. M. Richardson and Dr A. C. Fabergé. During the past two years cytological and also embryological studies have been carried out by Dr P. T. Thomas, who describes the results of his investigations in the accompanying paper.

REPRODUCTIVE BEHAVIOUR

(a) *Diploid species*

The results we have obtained in breeding experiments with *R. rusticanus* and its varieties *inermis* and *albus* are summarized in Table I.

The dominance of the prickled character and black fruit colour in the F_1 families 25/31 and 26/31 raised from reciprocal crosses between the varieties *inermis* and *albus* shows that reproduction in these families has been strictly sexual. The F_2 and backcrossed families raised from these varieties have not yet produced flowers or fruit, and consequently only the prickled character has so far been recorded. In these families there is

TABLE I

	♂ parent chromo- some no. 2n	Fruit colour		Prickles		Progeny chromo- some no. 2n	
		Black	Amber	Present	Absent		
<i>R. rusticanus</i>	14	+	.	+	.	14	
<i>R. rusticanus inermis</i>	14	+	.	.	+	14	
<i>R. rusticanus albus</i> *	14	.	+	+	.	14	
Fam. no.	Parents						
25/31	<i>inermis</i> × <i>albus</i>	14	30	-	30	-	14
26/31	<i>albus</i> × <i>inermis</i>	14	30	-	30	-	14
33/35	25-5/31 × 25-4/31	14	-	-	18	12	14
38/35	25-5/31 × 25-4/31	14	-	-	145	57	14
42/36	<i>inermis</i> × 25-1/31	14	-	-	68	105	14
43/36	<i>inermis</i> × 26-6/31	14	-	-	65	111	14
44/36	<i>albus</i> × 25-17/31	14	-	-	85	-	14
45/36	<i>albus</i> × 26-5/31	14	-	-	18	-	14
11/12	<i>inermis</i> × <i>R. laciniatus</i>	28	1	-	1	-	21
1/22	<i>inermis</i> × <i>R. thyrsiger</i>	28	1	-	4	-	(3) 21 (1) 28

* For convenience this form with yellow or amber coloured fruits is referred to throughout this paper as *R. rusticanus albus*.

an excess of the prickleless recessive plants, especially in the backcrossed families 42/36 and 43/36, but there is no reason to think that this is due to a departure from normal sexual reproduction. The cytology of the few plants raised from crossing *inermis* with *R. laciniatus* and *R. thyrsiger* is also in agreement with sexual reproduction. In an earlier publication (Crane & Darlington, 1927) it was shown that the exceptional plant with 28 chromosomes obtained from *inermis* crossed *thyrsiger* arose from a unreduced germ cell of the maternal parent.

We have crossed *R. rusticanus inermis* with a number of other species of *Rubus* but no viable seeds were formed.

The plants of *R. rusticanus inermis* and *albus* used in these experiments are self-sterile. The former has been repeatedly self-pollinated but no fruits or seeds formed. In family 25/31 (*inermis* × *albus*) twenty-six

plants were self-pollinated and tested for self-sterility. Fifteen plants entirely failed (self-sterile), eleven set occasional drupels (slightly self-fertile). In the reciprocal cross, family 26/31, twenty-four plants were tested. Twenty-one were self-sterile, two slightly self-fertile and one self-fertile. One example of cross-sterility was found in this family.

Rubus idaeus, $2n=14$. The data relating to this species are very considerable; they involve numerous families and several thousand individuals, and it is clear from the mode of inheritance of the various characters studied that reproduction in this species is also strictly sexual (see Crane & Lawrence, 1931; Lewis, 1939).

In both *R. rusticanus* and *R. idaeus* the occurrence and functioning of unreduced germ cells is not uncommon. For example, the Veitchberry ($2n=28$) and the mahdiberry ($2n=21$) were both raised from crossing *R. rusticanus* with *R. idaeus*, and the John Innes berry ($2n=28$) arose from non-reduction on the part of its female parent, *R. rusticanus*. The tetraploid forms of *R. idaeus* such as Belle de Fontenay, Merveille Rouge, Everbearing, Hailshamberry, etc., are autotetraploids and have probably arisen from the functioning of unreduced germ cells. These forms are comparatively recent and appear to have originated from diploids in the last eighty years. Autotriploid raspberries have also arisen from diploids in breeding experiments at Merton (Lewis, 1940). These examples demonstrate one of the methods by which polyploids originate.

Incidentally, I know of no diploid hybrid the result of hybridization between *R. rusticanus* and *R. idaeus*. It therefore seems that it is easier for fertilization to occur in conjunction with non-reduction, and possibly it is only when an unreduced germ cell, from one or both diploid parents, takes part in fertilization, or when a polyploid form of *R. idaeus* is one of the parents, that viable seeds are formed when these species are intercrossed. So far, attempts I have made to intercross these diploid species have entirely failed.

(b) *Polyploid species*

The results obtained from breeding experiments with *R. vitifolius*, the loganberry *R. loganobaccus*, and with diploid and tetraploid forms of *R. idaeus* are summarized in Table II.

The chromosome numbers ($2n=49$) of the plants in families 1/34 and 2/34 raised from reciprocal crosses between *R. vitifolius* ($2n=56$) and *R. loganobaccus* ($2n=42$) shows that they have arisen from normal sexual reproduction. Similarly, the chromosome number ($2n=35$) of the plants in family 3/34 raised from *R. vitifolius* ($2n=56$) crossed *R. idaeus* ($2n=14$)

shows that they are sexually reproduced. The morphological characters of these families detailed in Table III also agree with a strictly sexual origin.

TABLE II

Family no.	Parents			F_1				
		Sex	Chromosome no. $2n$	Sex			Chromosome no. $2n$	Mode of reproduction
				♀	♂	♂		
4/34	<i>R. vitifolius</i>	♀	56					
	×							
	<i>R. vitifolius</i>	♂	56	26	5	35	56	? Sexual
2/34	<i>R. vitifolius</i>	♀	56					
	×							
	Loganberry	♀	42	24	21	7	49	Sexual
1/34	Loganberry	♀	42					
	×			—	27	2	49	Sexual
	<i>R. vitifolius</i>	♂	56					
3/34	<i>R. vitifolius</i>	♀	56					
	×			21	19	—	35	Sexual
	<i>R. idaeus</i> *	♀	14					
	<i>R. vitifolius</i>	♀	56					
	×			—	10	—	42	Sexual
	<i>R. idaeus</i> *	♀	28					
	<i>R. vitifolius</i>	—	—					
	×			—	1	—	43	Sexual
	<i>R. idaeus</i> *	—	—					
	<i>R. vitifolius</i>	—	—					
	×			1	—	—	44	Sexual
	<i>R. idaeus</i> *	—	—					
7/34	<i>R. vitifolius</i>	—	—					
	×			—	1	—	50	?
	<i>R. idaeus</i> *	—	—					
	<i>R. vitifolius</i>	—	—					
	×			4	—	3	56	Asexual
	<i>R. idaeus</i> *	—	—					
	<i>R. vitifolius</i>	—	—					
	×			1	—	—	35	?
	<i>R. idaeus</i> *	—	—					
15/13	Loganberry (selfed)	♀	42	—	53	—	42	? Sexual
31/37	7/34 (17) (selfed)	♀	42	—	45	—	? 42	? Sexual

* The diploid form of *R. idaeus* used in the experiments was the variety Superlative, and the tetraploid the variety Hailshamberry.

In family 7/34 raised from *R. vitifolius* ($2n=56$) crossed to a tetraploid form of *R. idaeus* ($2n=28$) the plants fall into two groups, with 56, and with less than 56, chromosomes. The 56 chromosome plants are obviously of asexual (maternal) origin. Morphologically they are like the maternal parent *R. vitifolius* and have the same number of chromosomes. The ten plants with 42 chromosomes are evidently sexually reproduced. They

have the chromosome number expected from the cross between an octoploid and a tetraploid, and their morphology given in Table III also supports this view. The female organs of all are well developed; six are fully hermaphrodite in structure, but in the remaining four plants the male organs are not completely developed. The three plants with 43, 44, and 50 chromosomes also appear to be of sexual origin; probably as the result of union of normal reduced germ-cells of *R. vitifolius* ($n=28$) with unbalanced germ-cells from the tetraploid male parent with extra chromosomes. The plant with 35 chromosomes shows no trace of *R. idaeus*, the male parent, and therefore appears to be of asexual origin.

Segregation of well-defined genetic characters occurs within *R. vitifolius*. In this species the flowers are prevailingly unisexual, the large majority of plants being either entirely male or entirely female (see

TABLE III

	Chromosomes		Leaves
	No.	Proportion derived from raspberry	
Raspberry \times raspberry	$2x=14$	All	Five distinct leaflets
Raspberry \times raspberry	$4x=28$	All	" "
<i>R. vitifolius</i> $\varnothing \times R. vitifolius$ σ	$8x=56$	0	Three-lobed "
<i>R. vitifolius</i> \times loganberry	$7x=49$	1/7	Three-lobed but more deeply incised than <i>vitifolius</i>
<i>R. vitifolius</i> \times raspberry $2x$	$5x=35$	1/5	Three-lobed but more deeply incised than previous family
<i>R. vitifolius</i> \times raspberry $4x$	$8x=56$	0	Three-lobed
	$6x=42$	1/3	Five distinct leaflets
Loganberry	$6x=42$	1/3	Five distinct leaflets

family 4/34, Table II); in a few plants there is a slight development towards hermaphroditism, one sex being well developed and the other developed only to a slight degree. Such flowers are not, however, functionally hermaphrodite. Variation within the species also occurs with respect to pigmentation and the amount of waxy bloom on the growth, some being pruinose, others glaucous; and in other minor respects. The asexually reproduced plants in family 7/34 show segregation of characters within the limits of the variation found in the maternal species. As shown in Table II, some have female and others male flowers. They also vary in pigmentation and waxiness.

The leaves of *R. vitifolius* are three-lobed, and, as previously described, the sexes are borne separately, some plants being male and others female. In the raspberry, both diploid and tetraploid, and in the loganberry, the leaves are pinnate, having five leaflets when fully developed; and the

flowers are hermaphrodite. As shown in Table III, in the families raised from inter-crossing these *Rubi* there is a gradation from the three-lobed leaf to the pinnate leaf with five distinct leaflets, which is directly correlated with the proportion of chromosomes derived from the pinnate-leaved raspberry.

Gustafsson (1930-8) citing Petersen (1921) states that "all American species belonging to the subgenus *Eubatus* are sexual", but the behaviour of *R. vitifolius*, a species in this subgenus, shows that this generalization is not universally valid. As shown in Table II, when this species is crossed with the loganberry or the diploid raspberry reproduction is strictly sexual, but when crossed with the tetraploid raspberry a proportion of the offspring is produced sexually and a proportion asexually. It is

TABLE IV

Family	Parents, all tetraploids, $2n=28$	Sexual	Offspring, presumed asexual on cytological grounds	Asexual
7/32	<i>R. thyrsiger</i> , selfed	—	284	—
17/32	<i>R. thyrsiger</i> × <i>R. nitidioides</i>	2	—	32
5/32	<i>R. nitidioides</i> × <i>R. thyrsiger</i>	1	—	9
15/33	<i>R. thyrsiger</i> × <i>R. calvatus</i>	5	—	6
10/32	<i>R. calvatus</i> × <i>R. thyrsiger</i>	6	—	14
16/33	<i>R. thyrsiger</i> × <i>R. procerus</i>	2	—	4
34/37	<i>R. thyrsiger</i> × <i>R. gratus</i>	5	—	33
12/32	<i>R. calvatus</i> , selfed	—	73	—
13/33	<i>R. calvatus</i> × <i>R. procerus</i>	3	—	5
9/32	<i>R. calvatus</i> × (<i>R. rusticanus</i> × <i>R. thyrsiger</i>)	2	—	6
25/35	<i>R. Schlechtendalii</i> × <i>R. procerus</i>	7	—	2
27/32	<i>R. Schlechtendalii</i> × <i>R. thyrsiger</i>	9	—	8
6/32	<i>R. nitidioides</i> , selfed	—	28	—
30/37	<i>R. nitidioides</i> × <i>R. Schlechtendalii</i>	4	—	41

therefore evident that in *R. vitifolius* the form of reproduction depends on the male used in pollination.

The breeding experiments with *R. vitifolius* and the raspberry were originally undertaken with a view to elucidating the origin of the loganberry. It may be recalled that the loganberry was originally described as a natural hybrid. The parents were believed to be the Aughinbaugh, a form of *R. vitifolius* and a raspberry (see Bailey, 1923). Subsequently, for various reasons, this came to be disputed (Darrow, 1933; Hedrick *et al.* 1925). These investigations support the originally postulated hybrid origin of the loganberry. As shown in Tables II and III the cytology, genetics and morphology of the hexaploid hybrids in the families 7/34 and 31/37 substantiates the above view. Full details will be published in a later paper.

Table IV summarizes the results with respect to sexual and non-sexual

reproduction obtained in families from crosses between a number of European species of *Rubus*. Among the different crosses made the proportion of sexually produced offspring varies considerably. In some of the selfed families, detailed in Table IV, the offspring were very uniform, but in others segregation occurred. For example in family 7/32 *R. thyrsiger* (selfed) the leaflets of the parent plant were comparatively narrow and spaced and the inflorescence very long and lax, whilst about 8 % of the selfed seedlings had much broader and overlapping leaflets and a shorter and more compact inflorescence. From the behaviour of family 7/34, described earlier in this paper, it is evident that segregation is not necessarily a criterion of sexual reproduction, consequently pending embryological investigations it is not possible to say with certainty what form of reproduction, whether sexual or non-sexual, occurs in these selfed families.

In family 7/32 three plants occurred with slender growth and small leaves. They were also comparatively infertile. It was found that these plants had lost a chromosome, their number being $2n=27$. Apparently such forms are found in nature. They correspond with the systematists' var. *microphyllus*.

In some of the crossed families the asexual offspring were uniform and identical with their female parent, but in others segregation occurred. For example, in family 17/33 *R. thyrsiger* crossed *R. nitidioides* one of the asexual plants had broad overlapping leaflets and a compact inflorescence similar to the segregates which appeared in the selfed family from *R. thyrsiger*. Another example of segregation in families asexually reproduced occurred in family 5/32, *R. nitidioides* crossed *R. thyrsiger*. One of the nine asexual plants in this family develops leaves with seven leaflets instead of the normal five leaflets of *R. nitidioides*, it also has much larger flowers and a more compact inflorescence than its maternal parent. When selfed this plant breeds true. Alternatively since such a seven-leaflet type did not appear in the selfed family raised from *R. nitidioides* it is possible that its occurrence in family 5/32 may be due to a mutation, but it seems more probable that the first view is correct.

Lidforss (1914) described many cases of false and true hybrids in F_1 families raised from interspecific crosses in *Rubus*, and states that the false hybrids, i.e. the apomictic material offspring, were all alike. However, as I have described in this paper, in some of the families we have raised segregation occurred among the apomictic offspring.

PARTHENOGENESIS

During the course of these breeding investigations with *Rubus* a number of haploid plants have occurred. For example, a hexaploid plant we obtained under the name *R. Borreri*¹ gave, when selfed, a number of plants which were triploid or approximately so, their chromosome number varying from $2n=20$ to $2n=22$. Haploids have also occurred in breeding work with certain cultivated forms of *Rubus*. The Veitchberry ($2n=28$) crossed with the raspberry ($2n=14$) and with *R. rusticanus* ($2n=14$) gave families which were mostly triploids ($2n=21$) but a small proportion were diploids ($2n=14$).

PSEUDOGAMY

In four of the species used in the experiments, which have given asexual plants when pollinated with other species, a considerable number of flowers have been emasculated and isolated and left without pollination, but in no case did fruits or seeds develop. It is therefore evident that development is pseudogamous, pollination being essential for apomictic reproduction. This is in agreement with the findings of Gustafsson (1930) and Darrow & Waldo (1933).

PARTHENOCARPY

R. procerus, when selfed, set fruit freely, but no viable seeds developed. Externally the fruits, including the endocarp, appeared to be normal, but no true seeds were formed. Self-pollination is, however, necessary for the formation of these parthenocarpic fruits, as flowers of the species when emasculated and left unpollinated entirely fail. Natural seed of this species, presumably resulting from cross-pollination, gave plants identical with the female species, *R. procerus*.

SUMMARY OF REPRODUCTIVE TYPES

The genetic results show that the types of reproduction in *Rubus* which have occurred in these experiments can be classified as follows:

- (1) Sexual reproduction (general in diploid species).
- (2) Non-reduction at meiosis on female, male or both sides (*R. rusticanus*, *R. idaeus*).

¹ This plant of *R. Borreri* was exceptional, all other plants of *R. Borreri* examined were tetraploid ($2n=28$). Presumably the hexaploid form arose from the tetraploid by non-reduction.

(3)¹ Apomixis with segregation, presumably diploid parthenogenesis (*R. vitifolius*, *R. thyrsgiger*).

(4) Apomixis without segregation, presumably apospory (*R. calvatus*).

(5) Haploid parthenogenesis (*R. Borreri*, Veitchberry).

At the present stage of the investigations it is not possible to say whether the absence of segregation in class 4 is simply due to homozygosity or to exclusive aposporic reproduction which does not allow for segregation. There appears however to be a fundamental difference in the reproductive behaviour of classes 3 and 4, and this view is supported by the cytological and embryological studies of my colleague Dr P. T. Thomas as described in the following paper (pp. 122-3).

The results of the breeding experiments with diploid and polyploid species detailed in this paper are significant in view of the difficult taxonomy of *Rubus*. They show not only how new forms and species can arise, but also how they are able to maintain themselves in nature. Of the numerous species and micro-species of *Rubus*, many are evidently clones and subclones, produced by segregation or mutation, or both, and maintained by apomixis.

SUMMARY

1. The reproductive behaviour of a number of species and varieties of *Rubus* has been investigated.

2. Diploid species always behave sexually. Occasional unreduced germ-cells occur and take part in fertilization, giving rise to polyploid forms.

3. In polyploid forms and species reproduction may be entirely sexual, entirely non-sexual or partly sexual and partly non-sexual (apomictic).

4. Polyploid species vary in the degree to which apomixis is developed, and a particular species may show a variation in reproductive behaviour depending on the species used as male in cross-pollination.

5. Segregation has been found to occur within non-sexual offspring. Hence test crosses, in conjunction with cytological and embryological studies, are necessary to determine the precise mode of reproduction.

6. The results are discussed in relation to the difficult taxonomy of *Rubus*.

¹ The term apomixis is used here in the sense that there has been no intervention of the male germ-cells in reproduction. Alternatively in these cases reproduction may be by "automixis" (see Thomas, p. 123).

ACKNOWLEDGEMENTS

I am indebted to Dr H. M. Butterfield for seeds of *Rubus vitifolius* collected in California; from these seeds the plants of this species used in the experiments were raised. We are also indebted to Mr W. Watson of Bickley, Kent, for plants and seeds and for valuable assistance in the identification of British species of *Rubi*.

REFERENCES

- BAILEY, L. H. (1923). *Quidam Rubi Cultorum. Gentes Herbarum*, V. Pp. 155-56.
- CRANE, M. B. (1936). "Blackberries and hybrid berries." *R.H.S. Conference on Cherries and Soft Fruits*, pp. 121-8.
- CRANE, M. B. & DARLINGTON, C. D. (1927). "The origin of new forms in *Rubus*. I." *Genetica*, **9**, 241-78.
- (1932). "Chromatid segregation in tetraploid *Rubus*." *Nature, Lond.*, **129**, 869.
- CRANE, M. B. & LAWRENCE, W. J. C. (1931). "Inheritance of sex, colour and hairiness in the raspberry, *Rubus idaeus* L." *J. Genet.* **24**, 243-55.
- CRANE, M. B. & THOMAS, P. T. (1939). "Segregation in asexual (apomictic) offspring in *Rubus*." *Nature*, **143**, 684.
- DARROW, G. M. (1933). "Cytology and breeding of *Rubus macropetalus*, the Logan, and related blackberries." *J. agric. Res.* **47**, 315-30.
- DARROW, G. M. & WALDO, G. F. (1933). "Pseudogamy in blackberry crosses." *J. Hered.* **24**, 313-15.
- GUSTAFSSON, A. (1930). "Kastrierung und Pseudogamie bei *Rubus*." *Bot. Not.* **6**, 477-94.
- (1938). "Species formation and polyploidy within the apomictic genera *Rubus* and *Taraxacum*." *Genetics*, **23**, 149-50.
- HEDRICK, U. P. *et al.* (1925). *The Small Fruits of New York*. Albany, N.Y., p. 59.
- LIDFORSS, B. (1914). "Résumé seiner Arbeiten über *Rubus*." *Z. induct. Abstamm.- u. VererbLehre*, **12**, 1-13.
- LEWIS, D. (1939). "Genetical studies in cultivated raspberries. I. Inheritance and linkage." *J. Genet.* **38**, 367-79.
- (1940). "Relation between polyploidy and fruiting habit in raspberries." *Proc. 7th int. genet. Cong.* (in the Press).
- PETERSON, A. K. (1921). "Blackberries of New England." *Bull. Vt. agric. Exp. Sta.* **218**.

REPRODUCTIVE VERSATILITY IN *RUBUS*

II. THE CHROMOSOMES AND DEVELOPMENT

By P. T. THOMAS

John Innes Horticultural Institution, Merton

CONTENTS

	PAGE
The detection of apomixis	119
The mechanism of apomixis	120
Genetical implications	122
Apomixis in relation to polyploidy	124
Summary	127
References	128

THE DETECTION OF APOMIXIS

By combining somatic and meiotic chromosome studies in the progeny of crosses with embryological studies, apomictic forms of reproduction were detected among the species and crosses of *Rubus* described by Mr Crane in the previous paper of this series.

In the family obtained from the cross *R. vitifolius* \times *R. idaeus* ($2n=28$) the sexual hybrids could readily be detected by their intermediate chromosome number ($2n=42$). One plant was discovered, however, with 35 chromosomes; its exact mode of origin is difficult to determine. Meiosis could not be studied, since the plant, like the female parent, was unisexual (\varnothing). Morphologically, as Mr Crane has stated, it showed no evidence of its male parent. How such a plant might arise from the female parent alone is indicated by the abnormal spindle mechanisms which have been observed in *Pyrus*, *Prunus* and *Rubus* (Crane & Thomas, 1939; Thomas, unpublished). An apomictic pentaploid of this kind could by such means arise by union of a normal (28) with a super-reduced (7) germ cell within the same embryo-sac. From the cytological point of view, on the other hand, a super-reduction in the male parent is considered much more likely. The Hailshamberry is an autotetraploid of a type which gives a high proportion of univalents due to trivalent formation. These univalents are often cut off as a micro-pollen grain, and such a grain would probably be viable if it contained the seven haploid chromosomes of *R. idaeus*. Lewis (unpublished) has obtained diploid plants both from selfing tetraploid *R. idaeus* and from crossing it with diploid forms, which again indicates super-reduction in the tetraploids. The possibility of haploid parthenogenesis must not, however, be excluded in these cases.

It will be remembered that *R. vitifolius* ($8x$) \times *R. idaeus* ($2x$) gave only sexual progeny, which indicates that haploid pollen grains of *R. idaeus* have an advantage over diploid grains, and consequently any haploid grain produced in the tetraploid *R. idaeus* will probably function.

In the progeny of crosses between two species with the same chromosome number, for example *R. nitidioides* \times *R. thyrsiger*, family 5/32, it is only possible to detect apomixis cytologically by studying meiotic behaviour. Both these species behave cytologically as autotetraploids, and thus a sexual hybrid between them would be an allotetraploid whose behaviour would depend on the remoteness of the two parents. The sexual hybrid in family 5/32 showed only a slight lowering in the degree of quadrivalent formation, and it is concluded that the two species are relatively near related. Thus the hybrid is functionally an autotetraploid owing to the close affinity between the two parents.

Meiotic studies on the progeny of two closely related species having the same chromosome number can only be regarded, therefore, as confirmatory of the morphological evidence. Meiosis in the "mutant" seven-leaved apomictic plant from the progeny of the *R. nitidioides* \times *R. thyrsiger* cross was not studied, but pollen analysis showed only 25 % good pollen, whereas *R. nitidioides* itself and the remaining apomictic plants all had about 50 % good pollen. *R. thyrsiger* has 75 % good pollen, while the sexual hybrid had a pollen fertility slightly lower than *R. nitidioides*.

The mode of reproduction which obtains when a plant is selfed can only be satisfactorily determined by embryological study, for as Mr Crane has shown earlier, segregation of characters cannot be regarded as a criterion of sexual reproduction.

THE MECHANISM OF APOMIXIS

Preliminary embryological studies have been made on some of the species used in the crosses described by Mr Crane. The results, which will be published fully in a later report, show fundamental differences in behaviour which can be correlated with the breeding behaviour of the species concerned.

Rubus is not ideal material for cytological work, and satisfactory preparations could only be obtained after dissecting individual ovules from the carpels before fixation in La Cour's 2 BX¹ or 3/1 acetic/alcohol. I am indebted to Dr D. Eissler for assistance in dissecting and examining up to 500 ovules of each species. Sections were cut at 20 μ

¹ (Darlington & La Cour, 1940).

and the 2 BE fixations stained in gentian violet, while the acetic alcohol fixations were stained in aceto-carmin. Early stages stained better in gentian violet and later stages better in aceto-carmin. It was not possible to obtain good differentiation with aceto-carmin at the early stages, while the later stages stained with gentian violet were unsatisfactory owing to the presence of brownish globular inclusions which masked the stain.

R. rusticanus ($2n=14$) was studied as an example of a sexually reproducing species in order to compare its behaviour with that of those species which reproduce by apomixis. Meiosis occurs in the embryo-sac mother cell almost as soon as in the anthers. The embryo-sac forms very quickly, and the polar nuclei fuse to form the central fusion nucleus at least two days before the flower opens. No departure from normal development was observed in this species.

R. nitidioides ($2n=28$), on the other hand, shows such a degree of irregular behaviour that normal sexual development must be the exception rather than the rule. This is in agreement with the observed behaviour of this species when pollinated by *R. thyrsiger* ($2n=28$). The embryological studies show that aposporic development is the rule. There is, however, a considerable range in the time of degeneration of the sexual embryo-sac. In a large number of cases the sexual embryo sac did not degenerate until a fairly late stage of development, so that two embryo-sacs per ovule could often be observed.

In addition to aposporic development, certain abnormalities within the sexual embryo-sac are not uncommon. The young embryo-sacs may contain two nuclei at one end and only one at the other. This is presumably due to lack of time co-ordination of nuclear divisions at the two ends, such as has been observed in *Tulipa* (Newton, 1927; La Cour, unpublished). Furthermore, evidence has been obtained that the daughter nuclei of the nucleus which divides first may fuse again. The undivided nucleus then divides, and this may be followed by a division of the fused nucleus. Although the further development of the "diploid" nuclei has not yet been traced, it evidently provides another possible mechanism of apomictic reproduction.

R. vitifolius ($2n=56$). This species does not show the gross abnormal behaviour observed in the previous species, its mode of development being much more like that of the sexually reproducing *R. rusticanus*. In nearly every case the embryo-sac contained the normal number of nuclei at the late pre-fertilization stage and the presence of two embryo-sacs in one ovule was rare.

Abnormalities were observed, however, in the relative positions of

nuclei within the embryo-sac, and also in nuclear differentiation. The central fusion nucleus was often situated very near the egg cell and not in its normal central position. In a few instances this nucleus was situated even nearer to the micropyle than the egg nucleus. The significance of this observation is not clear, but it is of interest that Noack (1939) observed this situation only in aposporic embryo-sacs of *Hypericum*. There was no evidence in *R. vitifolius* that the embryo-sacs were aposporic, unless the sexual sac had degenerated at a very early stage.

Normally it is easy to distinguish the egg cell, by its position and characteristic vacuole, from the synergids, but in some cases this differentiation had not occurred. In other instances, two differentiated egg cells and one synergid could be seen at the micropylar end of the embryo-sac. It is difficult to trace the further development of such nuclei, but it is possible that an apomictic embryo could arise by fusion of haploid nuclei, whether differentiated or undifferentiated egg cells.

GENETICAL IMPLICATIONS

Mr Crane has shown that segregation of characters occurs even among the apomictic progeny of certain crosses in *Rubus*. For example, a form with seven leaflets was discovered among the apomictic progeny of the *R. nitidioides* \times *R. thyrsiger* cross. *R. nitidioides*, as stated above, generally reproduces by apospory, a method of reproduction which does not allow for segregation since the embryo is derived from a somatic cell.

The other mode of reproduction possible for this species, that of fusion of the two "haploid" nuclei within the embryo-sac, however, does allow for segregation, since the embryo would be derived from nuclei arising after the first division of meiosis. But nuclei within the embryo-sac are necessarily of the same genetic constitution, so that such an apomictic embryo would be homozygous. The seven-leaflet segregate in the above did breed true for this particular character on selfing, although some variation in the degree of pollen fertility was recorded. Proof of homozygosity could not be obtained, however, since in this case one cannot determine whether or not reproduction was wholly by apospory. Furthermore, "homozygosity" arising in an autotetraploid by fusion of identical nuclei in the embryo-sac does not mean that further segregation is entirely precluded. An autotetraploid which is triplex for a given gene can give quadruplex, duplex or nulliplex progeny, while a duplex will only give quadruplex or nulliplex.

Segregation among the *R. nitidioides* apomicts is very limited—only one "mutant" in a small family—but among the apomictic progeny in

R. vitifolius it is much more pronounced, and may perhaps occur in a different way: that suggested by Darlington (1932) in which segregation can occur by crossing-over followed by parthenogenetic development from a restitution nucleus. Such a restitution nucleus has not yet been observed in the ovule of *R. vitifolius*, although occasional examples were recorded in the anthers.

Should fusion of two egg cells, differentiated or undifferentiated, occur, as suggested by the embryological observations, the apomictic embryo would be "homozygous" in the same way as in *R. nitidioides*. Evidence that the embryo-sac in *R. vitifolius* is ready for either apomictic or sexual reproduction is provided by the fact that with haploid *R. idaeus* pollen reproduction is always sexual, whereas with diploid *R. idaeus* pollen, reproduction may be apomictic. The question arises, however, as to whether reproduction by fusion of haploid cells from the same mother cell really constitutes an apomictic mode of reproduction, since Winkler (1908) defines apomixis as reproduction without fusion of cells or nuclei, and Gustafsson (1935) regards as sexual any form of reproduction where there is fusion of two nuclei, whatever their origin. The distinction is important in considering the genetical implications of the different modes of reproduction.

We may therefore classify the various types of reproductive mechanisms as follows:

General classification	Embryology	Special description	Genetical expectation
Embryo formed by fusion of nuclei or cells } Amphimixis	<ul style="list-style-type: none"> Fusion of pollen and egg nuclei Fusion of nuclei derived from the same mother cell 	<ul style="list-style-type: none"> True sexual Automixis <ul style="list-style-type: none"> Fusion of immediate products of meiosis Fusion of haploid nuclei within the embryo sac 	<ul style="list-style-type: none"> Segregation Segregation Segregation giving only homozygous progeny
Embryo not formed by fusion of nuclei or cells } Apomixis	<ul style="list-style-type: none"> Embryo formed from diploid egg following non-reduction at meiosis Embryo formed from haploid egg following meiosis Embryo formed from haploid nuclei other than egg Embryo formed from diploid egg in embryo sac formed directly from somatic cell Embryo formed directly from nucellus 	<ul style="list-style-type: none"> Diploid parthenogenesis Haploid parthenogenesis Apogamy Apospory Nucellar embryony 	<ul style="list-style-type: none"> Segregation through crossing over Segregation Segregation No segregation Purely vegetative or clonal
	<ul style="list-style-type: none"> Gametophyte formed Gametophyte not formed 		

The distinction between apomixis and amphimixis on the basis of nuclear fusion is not altogether satisfactory. For example, there is no essential genetical difference between "fusion" of nuclei by non-reduction in diploid parthenogenesis and fusion of the immediate products of meiosis in automixis. We may therefore regard apomictic processes which allow for segregation as subsexual, although the progeny are wholly maternal.

APOMIXIS IN RELATION TO POLYPLOIDY

On the basis of cytological observations several authors have advanced the theory that apomixis is conditioned by or associated with heterozygosity and polyploidy (Rosenberg, 1917; Ernst, 1917, 1918; Winge, 1917). Gustafsson (1935) also concludes that "The genus *Taraxacum* furnishes another instance of parthenogenesis correlated with polyploidy and cytological hybridity". The precise implication of the latter term, however, is not clear. It is apparently applied wherever meiosis is disturbed to any degree, without reference to the cause of this disturbance. We are now in a position to classify the various meiotic disturbances more precisely and to attribute them to their causes. For example, meiosis may be disturbed in polyploids owing to lack of chromosome homology, on the one hand, or excess of chromosome homology in autopolyploids on the other. Either of these disturbances will lead to varying degrees of sterility.

Darlington (1932, 1939) has shown apomixis as an escape from sterility, whatever the cause of the sterility, and from this point of view therefore we may consider the relationship between chromosome behaviour at meiosis and the methods of reproduction. Among polyploids, to which apomixis is largely confined in the flowering plants, the form of sterility most commonly found is that due to autopolyploidy. In Table I I have therefore arranged some *Rubus* species in ascending order of alloploidy, i.e. of differentiation between complementary sets, ranging from autopolyploids with a high degree of multivalent association to alloploids which have their two sets of chromosomes so well differentiated that they behave as diploids. In this way we can see what relationship exists between (1) cytological, (2) embryological, and (3) genetical behaviour in these species.

As shown in Table I, the first three species, *R. Borreri*, *R. nitidioides* and *R. thyrsiger* can conveniently be placed in one group. They are all autopolyploids, all show a very high degree of abnormal female gametophyte development, and the two which have been studied genetically

(*R. thyrsiger* and *R. nitidioides*) reproduce almost exclusively by one kind of apomixis or another. Here then we have an apomictic mode of reproduction associated with autopolyploidy.

At the other extreme we have the allopolyploids, the John Innes berry (RT 4) and the loganberry, which so far as we know reproduce sexually like the diploid *R. rusticanus* (Crane & Darlington, 1927, 1932). The intermediate position of *R. vitifolius* is interesting on account of its chromosome constitution and its mode of reproduction. Cytologically this species behaves as a doubled allotetraploid of constitution $A_1A_1A_1A_1A_2A_2A_2A_2$, $B_1B_1B_1B_1B_2B_2B_2B_2$, etc. This is shown (i) by its

TABLE I

Evidence of reproductive mechanism

Species	Cytological	Embryological	Genetical
<i>R. Borveri</i> ($2n=42$)	Autohexaploid (haploid may show complete pairing)	Largely aposporous	Mixed, including haploids
<i>R. nitidioides</i> ($2n=28$)	Autotetraploid 5-6 ^{VI} (50 % good pollen)	Largely aposporous (90 %). Abnormal sexual embryo-sacs	5 % sexual, 95 % apomictic
<i>R. thyrsiger</i> ($2n=28$)	Autotetraploid 2-3 ^{VI} (75 % good pollen)	Largely aposporous (80 %). Abnormal sexual embryo-sacs	7 % sexual, 93 % apomictic
<i>R. vitifolius</i> ($2n=56$) ♀ and ♂	Autotetraploid, allo-octoploid	Mainly normal sexual development, low apospory. Some abnormal sexual embryo-sacs	× 4× <i>R. idaeus</i> , 66 % sexual × other species, 100 % sexual
John Innes (RT 4) <i>R. rusticanus</i> × <i>R. thyrsiger</i>	Allotetraploid	—	100 % sexual(?)
<i>R. loganobaccus</i> ($2n=42$)	Allohexaploid 21 ^{II}	Rare apospory	100 % sexual
<i>R. rusticanus</i> ($2n=14$)	Diploid	Normally sexual	100 % sexual

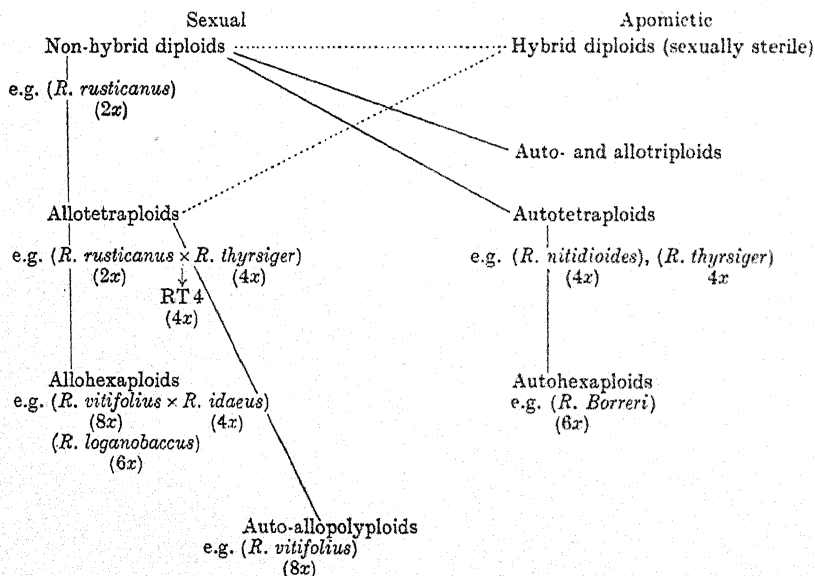
forming, itself, quadrivalents but not octavalents and (ii) by its showing complete bivalent autosyndesis in hybrids with other species (Thomas, 1940). The auto-allopolyploid constitution of *R. vitifolius* is reflected in its peculiarly versatile mode of reproduction, which in certain crosses takes the form of complete sexuality but in the cross with tetraploid *R. idaeus* results in about 40 % of asexual reproduction. Table I therefore sets out the transition from completely apomictic to completely sexual reproduction, passing through versatile stages, a transition which is correlated with a transition from autopolyploidy to allopolyploidy.

We have as yet no examples in *Rubus* of allopolyploids with very low chromosome homology, such as one could obtain by crossing two different

allotetraploids, or indeed of any type with complete absence of chromosome pairing. But regarding apomixis as an escape from sterility, as Darlington concludes, we should predict apomictic development in such plants, for sterility will arise as readily from too little pairing as from too much.

If we arrange our results as in Table II we find that the classification corresponds with Darlington's conclusion, the fertile allopolyploids being sexual and the less fertile autopolyploids and other unbalanced forms generally reproducing by apomixis.

TABLE II



It must be realized, however, that although apomixis is an escape from sterility, it is an escape conditioned by the genetic potentialities of the organism. Thus certain genera have a capacity for apomictic reproduction while others however sterile will not reproduce in this way. The difference between the two types is evidently genetical. The two sections of *Rubus* differ in this way. In the section *Ideobatus* there is no evidence that autotriploid and autotetraploid forms, e.g. of *Rubus idaeus*, reproduce by apomixis, although they are less fertile than the diploids (Lewis, 1940; also cf. *Pyrus* and *Sorbus*).

Gustafsson (1939) states that all the American species of *Eubatus*, in contrast to the European species, are sexual, but as shown above, our only example is an exception to this generalization.

Evidence that autopolyploidy is the immediate cause of apomixis in certain sections of *Rubus* is provided by the works of Lidforss (1914). By crossing pseudogamous species he showed that "im Gegensatz zu den falschen *Rubus*-bastarden zeigen die echten Bastarde dieser Gattung in F_2 immer weitgehende Spaltungen, die eine fast unbegrenzte Polymorphie der F_2 Generation bedingen". To take one example of many, he crossed various members of the aggregate *R. corylifolius* as female with *R. caesi*us and in addition to maternal seedlings obtained true F_1 plants which were intermediate between the two parents. Gustafsson (1938) finds the most of the *corylifolius* aggregate are tetraploids and Lidforss states that they show from 60 to 70 % of sterile pollen grains, so that they are evidently autotetraploids. Lidforss also states that *R. caesi*us shows a fairly high percentage of sterile pollen grains, and is also evidently an autotetraploid. The true F_1 hybrids, however, show 100 % of fertile pollen grains, and on selfing reproduce sexually to yield a variety of forms through segregation. Undoubtedly we have an example of a sexually reproducing allopolyploid produced by crossing two different pseudogamous autotetraploids, or diagrammatically **AAAA** (sterile and pseudogamous) \times **BBBB** (sterile and pseudogamous) = **AABB** (fertile and sexual).

SUMMARY

1. Apomictic modes of reproduction were detected in *Rubus* by combining somatic and meiotic studies with embryological studies.
2. Apospory, i.e. development from a purely vegetative embryo-sac, is the general method of non-sexual reproduction in the species examined.
3. Exceptional behaviour may occur within the sexual embryo-sac, such as (a) fusion of haploid nuclei, (b) presence of two differentiated eggs and one synergid or (c) presence of three undifferentiated nuclei at the micropylar end.
4. These abnormalities will account for the segregation of characters among asexual progeny which has been observed by Mr Crane (pp. 113, 114).
5. Comparison of the developmental processes with genetical behaviour in six polyploid species of *Rubus* shows that a transition from a completely sexual to completely apomictic mode of reproduction is correlated with a transition from allopolyploidy to autopolyploidy.
6. The condition determining apomixis in *Rubus* thus seems to be sexual infertility following autopolyploidy. The work of Lidforss, in which two different pseudogamic tetraploids when crossed always yield sexually reproducing allopolyploids, agrees with this view.

REFERENCES

- CRANE, M. B. & DARLINGTON, C. D. (1927). "The origin of new forms in *Rubus*. I." *Genetica*, **9**, 241-76.
- (1932). "Chromatid segregation in tetraploid *Rubus*." *Nature, Lond.*, **129**, 869.
- CRANE, M. B. & THOMAS, P. T. (1939). "Genetical studies in pears. I. The origin and behaviour of a new giant form." *J. Genet.* **37**, 287-99.
- DARLINGTON, C. D. (1932). *Recent Advances in Cytology*, 1st ed. London: Churchill.
- (1939). *The Evolution of Genetic Systems*. Camb. Univ. Press.
- DARLINGTON, C. D. & LA COUR, L. (1940). "The causal sequence of meiosis. III. The effect of hybridity on male and female cells of *Lilium*." *J. Genet.* **40**.
- ERNST, A. (1917). "Über den Ursprung der apogamen Angiospermen." *Vjschr. naturf. Ges. Zürich*, **62**.
- (1918). *Bastardierung als Ursache der Apogamie im Pflanzenreich*. Jena.
- GUSTAFSSON, A. (1930). "Kastrierung und Pseudogamie bei *Rubus*." *Bot. Not.*, **6**, 477-94.
- (1935). "Studies on the mechanism of parthenogenesis." *Hereditas, Lund*, **21**, 1-112.
- (1939). "Differential polyploidy within blackberries." *Hereditas, Lund*, **25**, 33-47.
- LEWIS, D. (1940). "Relation between polyploidy and fruiting habit in raspberries." *Proc. 7th int. genet. Cong.* (in the Press).
- LIDFORSS, B. (1914). "Résumé seiner Arbeiten über *Rubus*." *Z. indukt. Abstamm.- u. VererbLehre*, **12**, 1-13.
- NEWTON, W. C. F. (1927). "Chromosome studies in *Tulipa* and some related genera." *J. linn. Soc.* **47**, 339-54.
- NOACK, K. L. (1939). "Über *Hypericum*-Kreuzungen. VI. Fortpflanzungsverhältnisse und Bastarde von *Hypericum perforatum* L." *Z. indukt. Abstamm.- u. VererbLehre*, **76**, 569-601.
- THOMAS, P. T. (1940). "The origin of new forms in *Rubus*. III. The chromosome constitution of *R. loganobaccus* Bailey, its parents and derivatives." *J. Genet.* **40**, 141.
- ROSENBERG, O. (1917). "Die Reduktionsteilung und ihre Degeneration in *Hieracium*." *Svensk bot. Tidskr.* **11**.
- WINGE, O. (1917). "The chromosomes. Their numbers and general importance." *C.R. Lab. Carlsberg*, **13**.
- WINKLER, H. (1908). "*Solanum tubingense*, ein echter Propfbastard zwischen Tomate und Nachtschatten." *Ber. dtsh. bot. Ges.* **26**, 595-608.

THE ORIGIN OF NEW FORMS IN *RUBUS*

II. THE LOGANBERRY, *R. LOGANOBACCUS* BAILEY

By M. B. CRANE

John Innes Horticultural Institution, Merton

(With Plate V and One Text-figure)

CONTENTS		PAGE
Introduction		129
Material		131
Breeding experiments		132
Inheritance of sex		134
Dominance in polyploids		135
Review of the evidence of origin		136
Summary		139
Acknowledgement		139
References		139
Explanation of Plate V		140

INTRODUCTION

WHEN the loganberry, *Rubus loganobaccus* Bailey, was discovered sixty years ago it was assumed to be a new hybrid, the result of a cross between a blackberry and a raspberry. Subsequently, however, there has been much discussion as to whether it is really a hybrid or only a direct offshoot from the wild blackberry of California, *R. vitifolius*. This difference of opinion, and the conclusions of earlier work at Merton (Crane & Darlington, 1927), led me to undertake a series of breeding experiments to discover which of the above views is the more probable. Seeds of *R. vitifolius* were obtained from California, and in 1934 crosses were made between *R. vitifolius*, *R. idaeus* and other *Rubi*. The results of the breeding work are described and discussed in this report. Many of the plants raised in the experiments have been cytologically examined by my colleague, Dr P. T. Thomas, who gives an account of his studies in a separate paper (Thomas, 1940*b*).

The original story of the origin of the loganberry is probably best told in a communication from Judge J. H. Logan, in whose garden in California the loganberry was raised, to Prof. L. H. Bailey. This communication, quoted below, is dated 1902, and was published by Bailey (1923).

"In August 1881, I planted the seed of the common wild blackberry or dewberry, of California, botanically known as the *Rubus ursinus*,

gathered from plants on one side of which was growing a kind of evergreen blackberry known as the Texas Early, and on the other side of which was growing an old variety of red raspberry. The Texas Early has a growth of cane and leaves similar to the Lawton, although much less vigorous, and in our climate is growing winter and summer. It has a small round berry of more acidity than the Lawton and probably of poorer flavour. The raspberry referred to has been growing in this place for the last forty years and I am unable to ascertain what variety it is, although it is of a type similar to the Red Antwerp. It is not, however, the Red Antwerp as we have been growing it here. From this seed there grew about one hundred plants which were cared for and planted out in the ground. In the summer of 1883 these plants fruited and there appeared one plant which was undoubtedly a cross between the raspberry and the *Rubus ursinus*. The fruit was larger and earlier than the raspberry or any blackberry, except the *Rubus ursinus*, ripening about the middle of May; the appearance of the berry on the surface was something like the raspberry, being less indented and of more even surface than a blackberry; the colour a bright glowing red, becoming very dark and finally, when dead ripe, of a dull purplish-red colour. The berry has a core like the blackberry and parts from the calyx the same as a blackberry. The leaves of the vine are almost identical with the wild *Rubus*, being somewhat larger. The canes are also like the wild *Rubus*, only larger and more vigorous. It has the same small sharp spines and, like it, is without adventitious root buds, but multiplies from the stolons or tips or from seed. The fruit, when cooked, has the same rich acidity as the wild *Rubus*, there being only a suggestion of the taste of the raspberry in the cooked fruit, but in the jelly there is a more decided raspberry flavour. This red berry is universally known here as the loganberry."

The assumption of the hybrid origin of the loganberry went unchallenged for several years. Later, however, it came to be disputed, primarily because the high fertility of the loganberry seemed at that time inconsistent with the nature of a hybrid derived from widely distinct parents. Moreover, the types of habit, flower and fruit of the supposed parents failed to reappear in its seedlings, and again, on crossing with other *Rubi*, the dominance of many characters of the loganberry seemed more like the behaviour of a species than like that of a hybrid. For these reasons some authorities came to consider the loganberry as a variety of *R. vitifolius*, and rejected the postulated hybrid origin.

Bailey (1923) is strongly inclined to the opinion that the loganberry is a hybrid, as supposed in the beginning, and in his systematic studies

of the genus gives it specific rank as *R. loganobaccus*. Others, including Darrow (1937), Darrow & Longley (1933) and Hedrick (1925), favour the opposite view and conclude that it is not of hybrid origin.

Varieties of raspberries are of two kinds, the diploids and the tetraploids. The tetraploids have arisen from the diploids during the past eighty years. Morphological differences readily separate the two groups, and there is no doubt that the raspberry described by Logan as "of a type similar to the Red Antwerp", and thought by him to be the male parent of the loganberry, was one of the older diploids.

The species *R. vitifolius*, used in these experiments, is very closely allied to *R. ursinus* if not identical with it. They are both placed in the subgenus *Eubatus* section *Vitifolii*, and apparently their principal difference is one of hairiness. They are frequently confused and Focke (1914) reduces *vitifolius* to a variety of *ursinus*. Bailey (1923) gives a botanical description of *R. vitifolius* and follows with the statement: "I have not been able to identify any of the named cultivated dewberries with this species as here understood, although I suppose that the Aughinbaugh, once considerably planted in California, belongs here (or possibly to *R. ursinus*)." According to Darrow (1937) the Aughinbaugh is a selection of the wild blackberry or dewberry of California and it was from seed of the Aughinbaugh that the loganberry was raised.

MATERIAL

The following species were used in the experiments:

Species	Chromosome no. $2n$
<i>R. idaeus</i> L.	14
<i>R. idaeus</i> L.	28
<i>R. neglectus</i> Peck	14
<i>R. niveus</i> Thunb.	14
<i>R. loganobaccus</i> Bailey	42
<i>R. vitifolius</i> Cham & Schlecht.	56

The plants of *R. vitifolius* used in the experiments were raised from seeds collected in California by Dr H. M. Butterfield, who informed me that they came from an area where there was little danger of any natural hybridization. He also stated that the berries collected were of the rounded form and not so long as some found in certain other sections. The plants raised from these seeds were dioecious, being either strictly male or strictly female. Their fruits varied in shape; some were of the rounded form described by Dr Butterfield, others were longer than broad. Differences also occurred in the pigmentation and the amount of waxy bloom on the growth. Some had a heavy covering of bloom, whilst others

were free from it. On the young growth the leaves of all the plants were ternate.

The raspberries used in the experiments were the diploid variety "Superlative" and the tetraploid variety "Hailshamberry". As shown in recent papers (Crane, 1940; Thomas, 1940) the tetraploid raspberries are autotetraploid, and there is every reason for concluding that they have arisen directly from the diploids. Both varieties have hermaphrodite flowers and on the young canes the leaves are 5-pinnate.

The loganberry has hermaphrodite flowers and 5-pinnate leaves on the young growth.

BREEDING EXPERIMENTS

The results of the experiments, showing the sex, morphology, and chromosome number ($x=7$) of the parents and seedlings, are summarized in Table I.

TABLE I

Parents					Seedlings				
Family no.		Chromosome no.	Sex	Leaf division	Chromosome no.	Sex			% chromosomes from raspberry
						♀	♂	♂	
4/34	<i>R. vitifolius</i> × <i>vitifolius</i>	8x	♀	3	8x	26	5	35	0
2/34	<i>R. vitifolius</i> × Loganberry	8x	♀	3	7x	24	21	7	14
		6x	♂	5					
1/34	Loganberry × <i>R. vitifolius</i>	6x	♂	5	7x	—	27	2	14
3/34	<i>R. vitifolius</i> × raspberry	8x	♀	3	5x	21	19	—	20
		2x	♂	5					
7/34	<i>R. vitifolius</i> × raspberry	8x	♀	3	6x	—	10	—	33
31/37	7/34 17 (selfed)	4x	♂	5	6x	—	45	—	33
		6x	♂	5					
15/13	Loganberry (selfed)	6x	♀	5	6x	—	53	—	33
Many fams.	Raspberry × raspberry	2x	♀	5	2x	—	All*	—	100
		2x	♂	5					
	Raspberry × raspberry	4x	♂	5	4x	—	All	—	100
		4x	♂	5					

* As shown in previous papers (Crane & Lawrence, 1931; Lewis, 1939) sex forms occur in the raspberry, and as detailed in recent papers (Crane and Thomas, 1940) a proportion of the seedlings in Family 7/34 had fifty-six chromosomes and were of asexual origin and one had thirty-five chromosomes, but none of these occurrences essentially affect the investigation with which we are concerned. From the chromosome number, morphology and genetics of the seedlings detailed in Table I, it is concluded that they all arose by normal sexual reproduction.

In the leaf division columns in the table, 3=ternate and 5=5-pinnate leaves.

In the family 4/34, *R. vitifolius* ♀ × ♂, twenty-six of the seedlings had female flowers, thirty-five male flowers, and the flowers of five were

classed as hermaphrodite. In these five plants the female organs were normal, but the male were only partially or feebly developed. On the fruiting growth the leaves were lobed or ternate and on the young growth (turions) the leaves were ternate. Of the fifty-two plants in family 2/34, *R. vitifolius* × loganberry, twenty-four were female, twenty-one hermaphrodite and seven male. On the young growth the leaves were ternate but the leaflets were more deeply incised than in the *R. vitifolius* family 4/34.

In the reciprocal family 1/34, loganberry × *R. vitifolius*, twenty-seven seedlings were hermaphrodite and two male. The leaves were ternate and incised as in family 2/34. In family 3/34, *R. vitifolius* × diploid raspberry, twenty-one of the plants were female and nineteen hermaphrodite. The leaves on the young growth were ternate, but more deeply incised than those in families 1 and 2/34. As a rarity, an occasional plant in families 1, 2, 3 and 4/34 developed a leaf with five more or less distinct leaflets. The plants of sexual origin in family 7/34, *R. vitifolius* × tetraploid raspberry, were all hermaphrodite and the leaves on the young growth were 5-pinnate.

Family 31/37 was raised from selfing one of the sexually reproduced plants in family 7/34. All the plants in this family had hermaphrodite flowers, and 5-pinnate leaves on the young growth like their parent. The plants in family 15/13, loganberry selfed, were all hermaphrodite with 5-pinnate leaves.

In breeding experiments at Merton with tetraploid raspberries, including the Hailshamberry, the seedlings have all had hermaphrodite flowers and 5-pinnate leaves on the young growth. In the diploid raspberries, however, some varieties are homozygous for hermaphroditism and 5-pinnate leaves. Others are heterozygous and segregated for hermaphrodite, male and female flowers. The hermaphrodite and female forms have 5-pinnate leaves, but in the male forms the leaves have only three leaflets (see Crane & Lawrence, 1931; Lewis, 1939).

In all the above families segregation occurred for such characters as pigmentation of growth and prickles, waxy bloom on the stems, hairiness and other minor characters, and in family 4/34, *R. vitifolius* ♀ × ♂, the length of the fruit varied considerably. In the heptaploid families 1 and 2/34 and the pentaploid family 3/34, the plants were highly sterile, and in consequence the fruits formed were usually imperfect.

Families were also raised from crossing the loganberry with *R. neglectus* and *R. niveus*. As shown in an earlier paper (Crane & Darlington, 1927) these hybrids were extremely sterile.

Inheritance of sex

As previously mentioned, in family 4/34, *R. vitifolius* ♀ × ♂, the five plants classed as ♀ were intersexes rather than true hermaphrodites; the female organs were normal, but those of the male were only slightly developed. If these five plants are included in the female class we have 31 ♀ to 35 ♂, which approximates to a 1 : 1 ratio.



Text-fig. 1. A=female flower of *R. vitifolius*. B=male flower of *R. vitifolius*. C=hermaphrodite flower of 6x hybrid. These flowers are from three seedlings of family 7/34 (*R. vitifolius* 8x × *R. idaeus* 4x). A and B are 8x apomictic segregates, and C is 6x and of sexual origin.

In the family raised from *R. vitifolius* ♀ × loganberry ♂, there were approximately equal numbers of ♀ and ♂ plants, plus a few males. In the reciprocal cross, where *R. vitifolius* was used as the ♂ parent, no females occurred, two were males and the remaining twenty-seven were hermaphrodites. Again when *R. vitifolius* ♀ was crossed with diploid raspberry, ♂, one-half of the seedlings were female and one-half hermaphrodite. The reciprocal cross, diploid raspberry × *R. vitifolius*, entirely failed. When *R. vitifolius* ♀ was crossed with the tetraploid raspberry, ♂, family 7/34, the sexual offspring were all ♀ (see Text-fig. 1).

As described in an earlier paper (Crane, 1940), a number of plants occurred in family 7/34 which were of asexual (apomictic) origin. These plants were typically *R. vitifolius*, like their mother, but some were female and some were male (see Text-fig. 1). This strongly suggests that in *R. vitifolius* the female is the heterozygous sex.

Dominance in polyploids

In polyploid plants, including *Rubus*, the phenomenon of dominance is often obscure and genetic analysis difficult owing to the intergradation of characters. Dominance is used here to mean character dominance and presumably depends on complex reactions rather than strict single gene reactions. When, however, we know the chromosome constitution of parental forms, and particularly when we know how the parents themselves have arisen, we begin to understand why the expression of dominance is essentially variable in polyploids. In this connexion the inheritance of the leaf characters of *Rubus* studied in these experiments is of interest. As shown in Table I, there is a gradation from the ternate leaf to the pinnate leaf with five distinct leaflets. If we accept the hybrid origin of the loganberry, we then find, as shown in the last two columns of the table, that this gradation is directly correlated with the proportion of chromosomes derived from the pinnate-leaved raspberry. Thus in hybrids where one-seventh of the chromosomes are derived from the 5-pinnate-leaved raspberry and six-sevenths from the ternate-leaved *R. vitifolius*, the hybrid plants are ternate, but the leaves are more deeply lobed than those of *R. vitifolius*. When one-fifth of the chromosomes are derived from the raspberry the hybrids are still ternate, but the leaves are more deeply incised than in the one-seventh case. With one-third raspberry chromosomes, the leaves of the hybrid are 5-pinnate like those of the raspberry. Other examples of intergradation of characters and change of dominance correlated with the proportion of parental chromosomes have occurred in our breeding work with other species of *Rubus*. For example (Crane & Darlington, 1927), when we crossed the widely different species *R. rusticanus* $2x$ with *R. thyrsiger* $4x$, most of the F_1 were $3x$ and very similar to their $4x$ male parent. One of the F_1 seedlings, however, was $4x$, the result of an unreduced germ-cell of its $2x$ parent taking part in fertilization. This plant, with fourteen instead of seven *rusticanus* chromosomes, was very similar to its $2x$ female parent.

REVIEW OF THE EVIDENCE OF ORIGIN

If the loganberry is a hybrid as originally supposed, it should be possible to remake it by means of suitable crosses between the presumed parents, and to make such crosses was the purpose of these experiments.

Before reviewing the results of the experiments, it may be of value to recall the principal characters of the species and forms used in the breeding work.

The leaves of *R. vitifolius* are ternate, and the sexes are borne separately, some plants being male and others female. The fruits are generally small and variable in shape. Some plants have a rounded form of fruit, whilst others have fruits which are much longer than broad. The somatic chromosome number of the species is 56.

In the loganberry the leaves are 5-pinnate, the flowers hermaphrodite and the fruits large and elongated. The somatic chromosome number is 42. There are therefore very pronounced differences between *R. vitifolius* and the loganberry in sex, morphology and chromosome number. These differences in themselves make it difficult to accept the view that the loganberry arose directly from *R. vitifolius*.

In the raspberry, *R. idaeus*, the leaves are 5-pinnate, the flowers hermaphrodite and the fruits comparatively large. The somatic chromosome number of most varieties is 14, a few being tetraploid, $4x=28$. We therefore see that the loganberry and raspberry, although different in chromosome number, are alike in their sexual condition and in the morphology of their leaves.

The plants in family 3/34, *R. vitifolius* \times diploid raspberry, are in several respects different from the loganberry. They differ in leaf morphology and chromosome number and are highly sterile and unproductive of fruit. Nevertheless, in most characters they are intermediate between their parents. In family 7/34, *R. vitifolius* \times tetraploid raspberry, however, the leaves of the plants of sexual origin are 5-pinnate, the flowers are hermaphrodite, fertile and productive of fruit, and the fruits of some are large and elongated. Their somatic chromosome number is 42. We therefore see that in all these major characters the hybrids are like the loganberry.

The fruits of the seedlings are not quite so large as those of the loganberry, but this I think is readily accounted for on the assumption that the named variety of *R. vitifolius*, "The Aughinbaugh", had larger fruits than the seedlings of *R. vitifolius* used in these experiments. As previously mentioned, the Aughinbaugh, the mother of the loganberry,

was selected from the wild and the size of the fruit would undoubtedly have been a major consideration in the selection. The F_2 , family 31/37, raised from *R. vitifolius* \times tetraploid raspberry, bred true in respect of the sex of the flowers and the 5-pinnate leaves, and there is no approach to the parental forms. Variation in size of fruit is of a similar order to that in families I have raised from selfing the loganberry. The plants in this family which have been examined by Dr Thomas are also hexaploid, $6x=42$, like the loganberry. All these facts agree with the originally postulated hybrid origin, and at the same time they contradict the view that the loganberry could have arisen as a direct offshoot of the wild blackberry of California.

There is no doubt that the raspberry similar to Red Antwerp, which was supposed by Judge Logan to be the male parent of the loganberry, was a diploid. We have seen from family 3/34 that *R. vitifolius* \times diploid raspberry gives pentaploid plants which are highly sterile and morphologically unlike the loganberry. When a tetraploid raspberry is used, however, the plants are hexaploid, fertile, productive of fruit and also morphologically like the loganberry. It is therefore reasonable to assume that an unreduced germ cell of the diploid raspberry took part in fertilization to give rise to the loganberry. The loganberry, that is, with $6x=42$, has twenty-eight chromosomes derived from *R. vitifolius* and fourteen derived from the raspberry. Many cases are on record of unreduced germ cells of raspberries and other *Rubi* taking part in fertilization and giving rise to new fertile forms (Crane & Darlington, 1927). One of these, the Veitchberry $4x=28$, was raised from *R. rusticanus* $2x=14$, \times raspberry, and like the loganberry it breeds nearly true, and in our breeding work at Merton unreduced germ cells of the raspberry have frequently taken part in fertilization (Lewis, unpublished).

One, and perhaps the most prominent, objection which has been advanced against accepting the postulated hybrid origin of the loganberry is that it is highly fertile and breeds nearly true; that is to say, although its seedlings are variable there is no approach to either parental form. Such an objection is untenable, and at the present time calls for no elaborate discussion. It is of course true that in hybrids from widely distinct forms, such as the parents of the loganberry, we are more familiar with the occurrence of sterility than of fertility, and when hybrids are fertile there is commonly much diversity in the progeny and an approach to the parental forms. But during the last decade, as a result of worldwide genetical and cytological research, we have become acquainted with many virtually true-breeding new forms and species which have

arisen from hybridization accompanied by chromosome duplication (see Crane & Lawrence, 1938; Crane, 1940). In many cases hybridization has been accompanied by complete duplication of the chromosomes to give rise to the new species, but in some cases the new species have arisen from hybridization and unilateral chromosome duplication, and this is presumably what occurred in the case of the loganberry, where the raspberry parent may be taken to have furnished a non-reduced germ cell.

In repeating the cross, if only the diploid raspberry had been available, we should have had to wait until one of the rare unreduced germ cells took part in fertilization; but as the raspberry already exists in the tetraploid as well as in the diploid form, it was possible to use a tetraploid variety in the cross and so provide an unlimited number of diploid germ cells. This, as we have seen, solved the problem. As the details in Table I and the figures in Pl. V show, the cross *R. vitifolius* \times tetraploid raspberry gave hybrids which in morphology, chromosome number, sex and fertility closely match the loganberry; and like the loganberry they breed practically true. The fact that their fruit size does not quite range up to that of the loganberry does not detract from the plausibility of the hybrid view; rather it adds to it, for, as mentioned earlier, one would naturally suppose that the cultivated variety of *R. vitifolius* grown by Logan to have had larger fruits than those of the wild seedlings we used, and to have handed on large size to their hybrid progeny.

Darrow & Longley (1933) have raised other objections and made some highly speculative suggestions. First they suggest that *R. loganobaccus* is a haploid species derived from *R. ursinus* and *R. macropetalus*, but since *R. ursinus* is $8x$, and *R. macropetalus* $12x$, it is difficult to see how the loganberry which is $6x$ could have arisen in such a way from hybridization between these two species. They conclude, however, that the loganberry arose from a wild form which they have themselves designated as *R. loganobaccus*, but they state: "The Logan differs from the wild *R. loganobaccus* in its hermaphrodite flowers and red fruit." There is, therefore, a gross difference between *R. loganobaccus* as described by Darrow & Longley and the loganberry, *R. loganobaccus* Bailey. The former, as defined, is prevalingly unisexual, whereas the loganberry is hermaphrodite, and this and the difference in fruit colour, as we have seen, are two of the principal characters which separate the loganberry from *R. ursinus* and its allied species or variety *R. vitifolius*. It does not seem necessary to pursue this question.

The whole series of blackberry-raspberry crosses we have made at Merton, in which varying proportions of raspberry chromosomes are

included, show behaviour which is in complete agreement with the hybrid origin of the loganberry, and there can now be no doubt that the loganberry is a hybrid as originally supposed by the raiser, Judge J. H. Logan.

SUMMARY

1. Although the loganberry arose as recently as 1881, the details of its origin are in dispute. It has been held (1) that the loganberry is a hybrid, the result of a cross between a blackberry and a raspberry, and (2) that it is not a hybrid but a "direct derivative" of the wild blackberry or dewberry of California, *Rubus vitifolius*. The present experiments were planned to decide between the two theories.

2. From crossing *R. vitifolius*, 8x, with *R. idaeus*, 4x, a hybrid was obtained which in morphology, chromosome number, sex and fertility closely match the loganberry; like the loganberry it is hexaploid and nearly true-breeding.

3. Crosses made between *R. vitifolius*, *R. idaeus*, 2x, and the loganberry, in which *R. vitifolius* and *R. idaeus* chromosomes were brought together in different proportions, gave results which are also in agreement with the hybrid view.

4. The only conclusion which can be reached from the experiments is that the loganberry is a hybrid as originally supposed, derived from an unreduced male germ cell of a raspberry, which is known to correspond to the diploid type, and a normal reduced germ cell of the blackberry.

5. The breeding data suggest that in *R. vitifolius* the female is the heterozygous sex.

6. The effects of introducing different proportions of parental chromosomes into hybrids are correlated with differences in morphological and other characters. This results in the intergradation of characters, a common feature of polyploids, and sometimes in a change of dominance.

ACKNOWLEDGEMENT

I am indebted to Mr A. Gavin Brown for assistance in the breeding work.

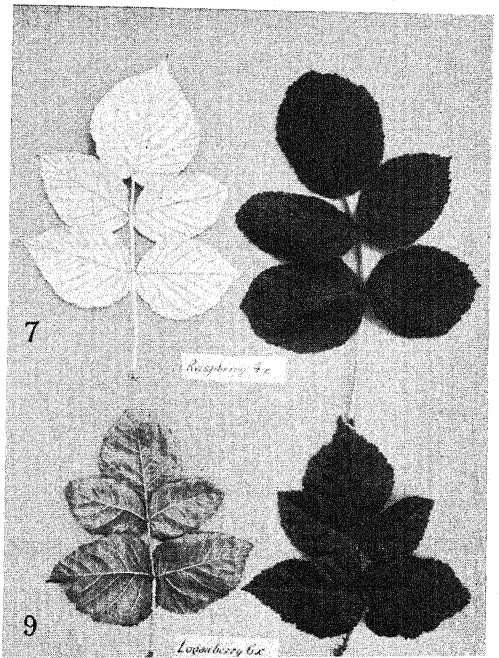
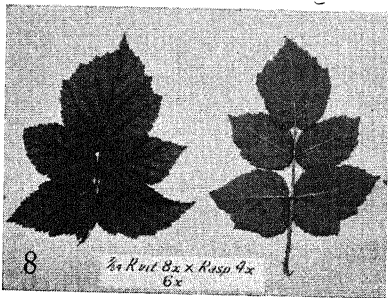
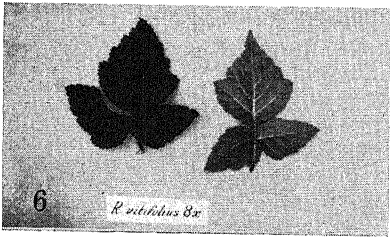
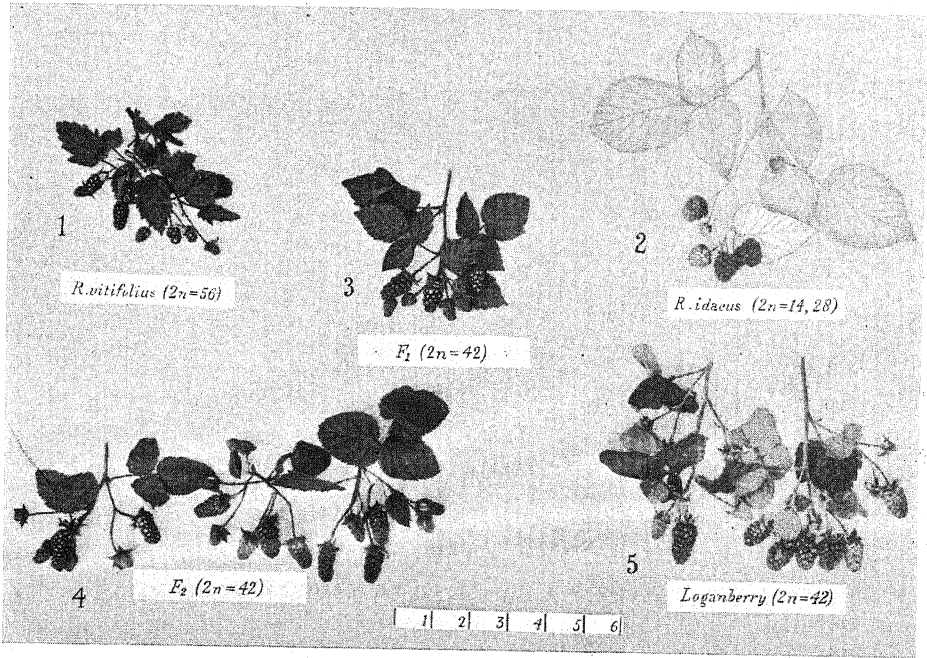
REFERENCES

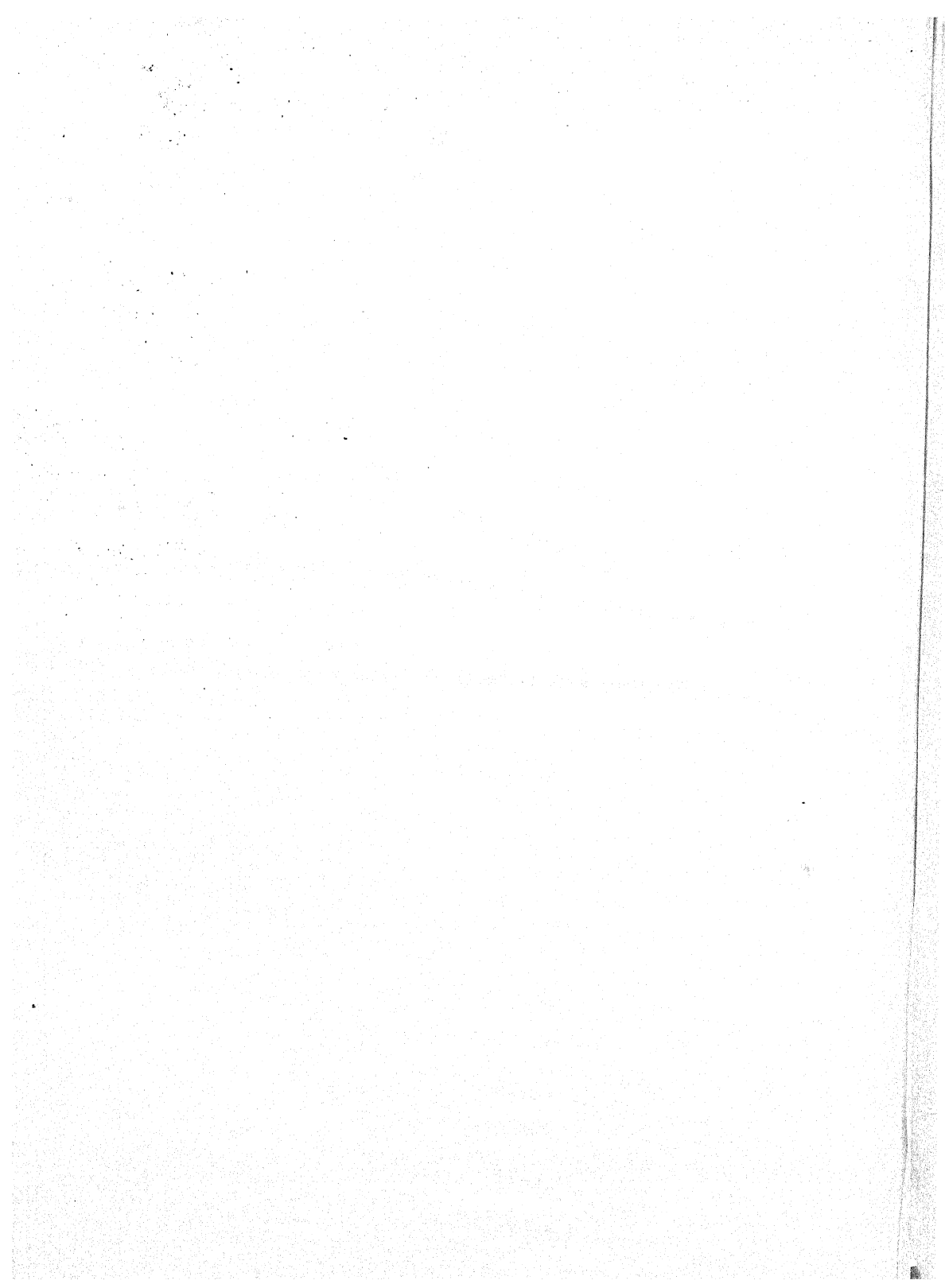
- BAILEY, L. H. (1923). "Quidam Rubi Cultorum." *Gentes Herbarum*, 5, 152-8.
CRANE, M. B. (1940). "Reproductive versatility in *Rubus*, I." *J. Genet.* 40, 109.
— (1940). "The origin and behaviour of cultivated plants." *The New Systematics* (ed. J. S. Huxley). Oxford.
CRANE, M. B. & DARLINGTON, C. D. (1927). "The origin of new forms in *Rubus*. I." *Genetica*, 9, 241-78.

- CRANE, M. B. & LAWRENCE, W. J. C. (1931). "Inheritance of sex, colour and hairiness in the raspberry, *Rubus idaeus* L." *J. Genet.* **24**, 243-55.
- (1938). *The Genetics of Garden Plants*, 2nd ed. London: Macmillan.
- DARROW, G. M. (1937). "Blackberry and raspberry improvement." *Yearb. U.S. Dep. Agric.* pp. 496-533.
- DARROW, G. M. & LONGLEY, A. E. (1933). "Cytology and breeding of *Rubus macro-petalus*, the logan and related blackberries." *J. agric. Res.* **47**, 315-30.
- FOCKE, W. O. (1914). *Bibl. bot., Stuttgart*, heft 83, 79.
- HEDRICK, U. P. (1925). *The Small Fruits of New York*. Albany, N.Y.
- LEWIS, D. (1939). "Genetical studies in cultivated raspberries. I. Inheritance and linkage." *J. Genet.* **38**, 367-79.
- THOMAS, P. T. (1940a). "Reproductive versatility in *Rubus*. II." *J. Genet.* **40**, 119.
- (1940b). "The origin of new forms in *Rubus*. III." *J. Genet.* **40**, 141.

EXPLANATION OF PLATE V

- Fig. 1. Fruits of *R. vitifolius*, showing round and elongated forms.
- Fig. 2. Fruits of *R. idaeus*.
- Fig. 3. Fruits of F_1 6x hybrid from *R. vitifolius* 8x \times *R. idaeus* 4x.
- Fig. 4. Fruits of F_2 from *R. vitifolius* \times *R. idaeus* 4x.
- Fig. 5. Fruits of *R. loganobaccus*.
- Fig. 6. Leaves of *R. vitifolius*.
- Fig. 7. Leaves of *R. idaeus* 4x.
- Fig. 8. Leaves of F_1 6x hybrid from *R. vitifolius* 8x \times *R. idaeus* 4x.
- Fig. 9. Leaves of *R. loganobaccus*.





THE ORIGIN OF NEW FORMS IN *RUBUS*

III. THE CHROMOSOME CONSTITUTION OF *R. LOGANOBACCUS* BAILEY, ITS PARENTS AND DERIVATIVES

By P. T. THOMAS

John Innes Horticultural Institution, Merton

(With Plate VI and Thirteen Text-figures)

CONTENTS

	PAGE
I. Introduction	141
II. Methods	142
III. Cytological behaviour	143
(1) <i>R. vitifolius</i> (8x)	143
(2) <i>R. idaeus</i> (2x, 3x, 4x)	143
(3) <i>R. vitifolius</i> (8x) × <i>R. idaeus</i> (2x)	144
(4) <i>R. vitifolius</i> (8x) × <i>R. idaeus</i> (4x)	145
(5) <i>R. loganobaccus</i> (6x) (loganberry)	145
(6) <i>R. loganobaccus</i> (6x) × <i>R. vitifolius</i> (8x)	146
(7) <i>R. loganobaccus</i> (6x) × <i>R. idaeus</i> (2x)	147
(8) <i>R. loganobaccus</i> (6x) × <i>R. neglectus</i> (2x)	147
(9) <i>R. loganobaccus</i> (6x) × <i>R. niveus</i> (2x)	147
IV. The autopoloidy of tetraploid raspberries	148
V. The auto-allopoloidy of <i>R. vitifolius</i>	148
VI. The origin and constitution of the loganberry	149
VII. The relationship between <i>R. vitifolius</i> and <i>R. idaeus</i> chromosomes	151
VIII. Chromosome relationships in other loganberry hybrids	151
IX. Supposed objections to the hybrid origin of the loganberry	153
X. Summary	154
References	155
Explanation of Plate VI	156

I. INTRODUCTION

THE loganberry is supposed to have arisen as a hexaploid hybrid by fusion of a diploid raspberry gamete with a tetraploid gamete from *R. vitifolius*. In order to test this view we first need to understand the nature of *R. vitifolius*.

The easiest method of deciding on the gametic constitution of this species would be of course to obtain a haploid with four sets of chromosomes. We can, however, obtain the same evidence by various indirect means. We can cross *R. vitifolius* with other species having lower chromo-

some numbers. Of such hybrids a variety are available from Mr Crane's experiments at Merton. By comparing the chromosome behaviour in *R. vitifolius* itself with that in each of the other species, diploid, tetraploid and hexaploid, I shall attempt to show how the four sets of chromosomes in the gametic complement of *R. vitifolius* are related to one another.

In the same way it is necessary to determine the chromosome constitution of the diploid raspberry gametes which were used in crossing with *R. vitifolius* (Crane, 1940 *a* and *b*). Crane & Darlington (1927) suggested that the diploid raspberry gamete involved in the production of the loganberry arose by non-reduction in a diploid, in which case the two sets of seven chromosomes would be identical. We have thus to discover whether the tetraploid raspberries which were used in the above experiments yield gametes of this constitution.

With such knowledge of the constitution of the two parental chromosome complements, it should be possible to show conclusively whether the loganberry arose as a hybrid or not.

The following species and hybrids used in the breeding experiments were studied cytologically:

<i>R. vitifolius</i>	8x
<i>R. idaeus</i>	2x, 3x, 4x
<i>R. vitifolius</i> × <i>R. idaeus</i>	(2x)	5x
<i>R.</i> „ × <i>R. idaeus</i>	(4x)	6x
<i>R. loganobaccus</i>	6x
<i>R.</i> „ × <i>R. vitifolius</i>	7x
<i>R.</i> „ × <i>R. idaeus</i>	(2x)...	4x
<i>R.</i> „ × <i>R. neglectus</i>	(2x)	4x
<i>R.</i> „ × <i>R. niveus</i>	(2x)...	4x

II. METHODS

Meiosis was studied after fixation of anthers in mixtures of absolute alcohol and glacial acetic acid varying from 1/1 to 5/1. Fixation was satisfactory in all these solutions, though the chromosomes were unduly swollen in the high acetic solutions. The pollen mother cells were stained in a dilute solution of acetocarmine to which an appreciable amount of iron acetate had been added. The chromosomes do not take up the stain readily, and it was found that the material fixed in the high-acetic solutions stained better than the other material.

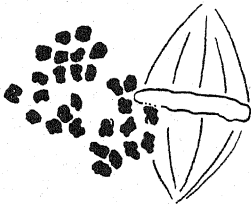
III. CYTOLOGICAL BEHAVIOUR

(1) *R. vitifolius* (8x)

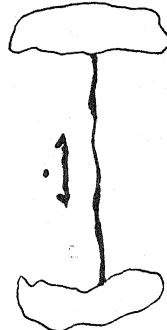
Rubus vitifolius, the western trailing dewberry, is an octoploid species (Text-fig. 1). At meiosis quadrivalents, bivalents and occasional trivalents and univalents are formed. Pl. VI, fig. 1 illustrates three quadrivalents at diakinesis (one linear and two ring types) in this species.



Text-fig. 1.



Text-fig. 2.



Text-fig. 3.



Text-fig. 4.

Text-fig. 1. Somatic chromosomes of *R. vitifolius* ($2n=56$). ($\times 2500$.)

Text-fig. 2. Metaphase II in *R. vitifolius*. ($\times 2000$.)

Text-fig. 3. Anaphase I showing bridge and fragment in *R. vitifolius*. ($\times 2000$.)

Text-fig. 4. Metaphase I in auto-triploid *R. idaeus*. ($\times 2000$.)

Owing to the large number of chromosomes it was not possible to analyse many complete nuclei, but it can be stated that from five to seven quadrivalents per nucleus are normally formed. Text-fig. 2 illustrates a polar view of one of the plates at second metaphase, showing the reduced number of chromosomes (28). Separation of configurations at first anaphase is generally complete, although there are bridges and fragments (Text-fig. 3), indicating heterozygosity for inversions, and even restitution nuclei in some cells.

(2) *R. idaeus* (2x, 3x, 4x)

The diploid forms of *R. idaeus* examined constantly form seven bivalents at meiosis.

The triploid form of this species, kindly provided by Dr Lewis, was a sterile auto-triploid obtained from diploids. Text-fig. 4 illustrates paired

configurations at metaphase, and Table I shows chromosome association at this stage from analysis of sixty complete nuclei.

TABLE I

	Trivalents	Bivalents	Univalents	No. of nuclei
	7	0	0	10
	6	1	1	13
	5	2	2	19
	4	3	3	11
	3	4	4	5
	2	5	5	2
Total* for 60 nuclei	306	114	114	60 nuclei
Mean association	5.1	1.9	1.9	

* These totals are obtained by multiplying the figures in columns 1, 2 and 3 respectively with the corresponding number of nuclei in column 4.

Two varieties of tetraploid raspberries were examined, the Hailsham-berry and Everbearing. There was no essential difference in the degree of chromosome association in the two types, but since the Hailsham-berry was used in the breeding experiments, it was studied in greater detail. The following analysis for eleven complete nuclei in the Hailsham-berry shows a range of from one to six quadrivalents per nucleus at metaphase.

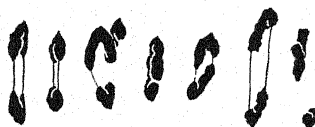
Text-fig. 5. Metaphase I in the Hailsham-berry, *R. idaeus* (4x). ($\times 2000$.)

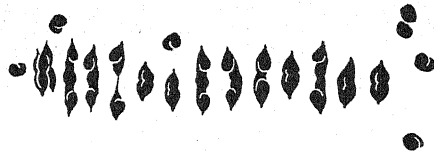
TABLE II

	Quadrivalents	Trivalents	Bivalents	Univalents	
	6	1	0	1	
	5	1	2	1	
	4	1	4	1	
	4	0	6	0	
	3	0	8	0	
	3	0	6	4	
	2	1	8	1	2 nuclei
	2	1	7	3	
	2	0	9	2	
	1	1	8	5	
Total for 11 nuclei	34	7	66	19	
Mean association	3.1	0.6	6.0	1.7	

(3) *R. vitifolius* (8x) \times *R. idaeus* (2x)

Text-figs 6 and 7 illustrate side views of paired and unpaired configurations at first metaphase in the pentaploid hybrid, and Table III shows the degree of association at this stage, from analysis of fourteen

complete nuclei. There is always complete association among twenty-eight of the thirty-five chromosomes in this hybrid, and since we find a lower number of univalents than seven, the seven extra chromosomes have also paired to some degree.



Text-fig. 6.



Text-fig. 7.

Text-figs. 6, 7. Metaphase I configurations from two nuclei in the hybrid
R. vitifolius \times *R. idaeus* ($2x$). ($\times 2000$.)

TABLE III

Quadrivalents	Trivalents	Bivalents	Univalents	No. of nuclei
1	0	12	7	3
1	1	11	6	1
—	1	13	6	4
—	2	12	5	2
1	3	9	4	1
—	3	11	4	1
1	4	8	3	2
Total for 14 nuclei	7	159	75	14 nuclei
Mean association	0.5	11.4	5.3	

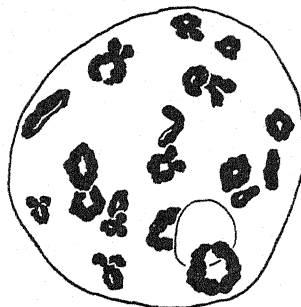
(4) *R. vitifolius* ($8x$) \times *R. idaeus* ($4x$)

The hybrid between *R. vitifolius* ($8x$) and *R. idaeus* ($4x$) is a hexaploid in which meiosis is almost completely regular. At first metaphase twenty-one bivalents are generally formed. Some quadrivalents have been observed (Text-figs. 8, 9) and even a few univalents. Disjunction of paired configurations is generally normal, although rarely bridges and fragments occur at first anaphase. The subsequent stages of meiosis are regular and about 60 % of the mature pollen grains germinate readily in culture (Pl. VI, fig. 6).

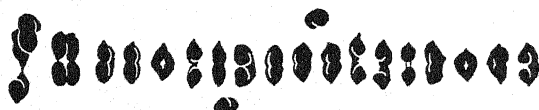
(5) *R. loganobaccus* ($6x$) : loganberry

R. loganobaccus has been investigated cytologically by Crane & Darlington (1927) and Darrow & Longley (1933). Some meiotic irregu-

larities were recorded by these investigators, but Darrow & Longley state that "the chromosomes were generally found to pair regularly, and the reduction phases show only occasional irregularities in chromosome distribution". They thus conclude that the plant behaves as a pure species.



Text-fig. 8.



Text-fig. 9.

Text-figs. 8, Diakinesis, and 9, Metaphase I in the hybrid *R. vitifolius* \times *R. idaeus* (4x).
($\times 2000$.)

My results confirm these observations. Association of chromosomes at metaphase was generally complete, but occasional cells showing two univalents occur at first metaphase (Pl. VI, fig. 5). The chromosomes normally pair as twenty-one bivalents and no cases of quadrivalent formation were observed.

(6) *R. loganobaccus* (6x) \times *R. vitifolius* (8x)

Owing to the complex nature of this heptaploid hybrid, analysis of complete nuclei at metaphase can only be made with difficulty. A range of from five to twelve univalents occur, the most usual number being about eight. Quadrivalents and trivalents are always formed, and the remaining chromosomes are associated as bivalents (Pl. VI, fig. 3).

Pl. VI, fig. 4 illustrates second metaphase in this hybrid and shows the orientation of undivided univalents at this stage. These univalents may sometimes orientate on the same spindle as one of the main daughter nuclei, thus causing a non-random distribution of chromosomes. The bottom right cell in this figure shows unequal distribution, in which there are twenty-four chromosomes at one pole while the remainder form two

groups—undivided univalents and products of pairing respectively—at the other pole.

(7) *R. loganobaccus* ($6x$) \times *R. idaeus* ($2x$)

Chromosome pairing at metaphase is by no means complete in this tetraploid hybrid. From four to nine univalents occur per nucleus. Some



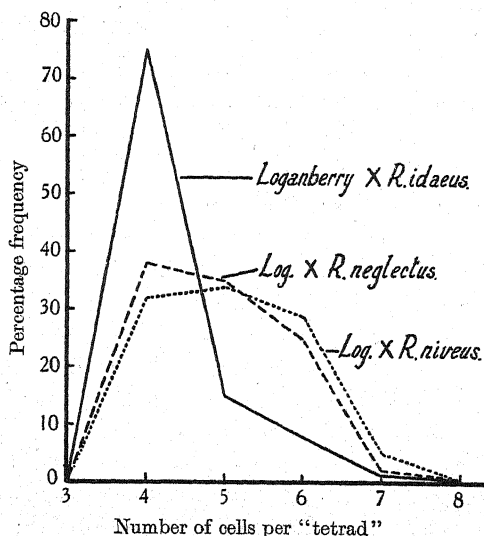
Text-fig. 10. Metaphase I configurations from one nucleus in the hybrid *R. loganobaccus* \times *R. idaeus* ($2x$). ($\times 2000$.)

trivalents are also found and the remaining configurations are bivalents (Text-fig. 10). There are always at least seven bivalents.

(8) *R. loganobaccus* ($6x$) \times *R. neglectus* ($2x$)

(9) *R. loganobaccus* ($6x$) \times *R. niveus* ($2x$)

These two tetraploid hybrids were studied at the later stages of meiosis only. The proportion of abnormal tetrads is in general a reliable criterion of the degree of meiotic irregularity in a plant which shows failure of pairing. Text-fig. 11 illustrates graphically the proportion of cases with 4, 5, 6 and 7 cells at the tetrad stage.



Text-fig. 11. Graph illustrating the frequency of abnormal tetrads in the hybrids *R. loganobaccus* \times *R. idaeus* ($2x$), *R. loganobaccus* \times *R. neglectus* and *R. loganobaccus* \times *R. niveus*.

The graph illustrates the difference in behaviour between *R. loganobaccus* \times *R. idaeus* ($2x$) on the one hand, and the hybrids *R. loganobaccus* \times *R. neglectus* and *R. loganobaccus* \times *R. niveus* on the other hand. Nearly 80 % of the tetrads in the cross with *R. idaeus* are normal, whereas in the crosses with *R. neglectus* and *R. niveus* the proportions are as low as 38 and 32 % respectively.

IV. THE AUTOPLOIDY OF TETRAPLOID RASPBERRIES

The only cytological studies available on tetraploid *R. idaeus* are those of Longley & Darrow (1924), who suggest that "polyploid raspberries are *Idaeobatus* \times *Eubatus* hybrids". These conclusions are based on the observation of meiotic irregularities, but, as is now known, such disturbances are evidence of precisely the contrary condition.

The present data (Tables II and IV) show that the Hailshamberry is an auto-tetraploid, and this applies equally to Everbearing. Quadri-valents are formed with an average of 3.1 per nucleus, and the presence of univalents is due to trivalent formation as well as to some failure of pairing.

In this connexion the tetrasomic segregation found by Lewis (unpublished) is also evidence of the auto-tetraploid nature of these raspberries.

V. THE AUTO-ALLOPLOIDY OF *R. VITIFOLIUS*

The chromosome constitution of *R. vitifolius* cannot be readily determined from its own behaviour at meiosis, since as many quadrivalents are expected in an auto-octoploid as in a doubled allo-tetraploid. But since no higher configurations than quadrivalents occur, the doubled allo-tetraploid constitution is favoured.

More precise information can be obtained from the behaviour of *R. vitifolius* \times *R. idaeus* ($2x$), since the hybrid will contain the four gametic sets of *R. vitifolius* chromosomes and one set from *R. idaeus*. We expect complete auto-syndesis among the *R. vitifolius* chromosomes whether they be of the constitution $v_1v_1v_1v_1$ or $v_1v_1v_2v_2$. We can conclude therefore that at least a very high proportion, if not all, of the univalents in this hybrid are *R. idaeus* chromosomes.

Regarding trivalent formation as being due to pairing of *R. idaeus* chromosomes, we find (Tables III, IV) that the twenty-eight *R. vitifolius* chromosomes form fourteen bivalents with an occasional quadrivalent. They thus virtually behave as a diploid of the constitution $v_1v_1v_2v_2$.

This view is confirmed by the behaviour of the hexaploid hybrid

R. vitifolius \times *R. idaeus* (4x) (Table IV). The chromosomes are completely paired, evidently owing to auto-syndesis among the gametic chromosome sets of both parents. The twenty-eight *R. vitifolius* chromosomes have behaved as they did in the *R. vitifolius* \times *R. idaeus* (2x) hybrid, by forming fourteen bivalents with an occasional quadrivalent.

TABLE IV

8x <i>R. vitifolius</i> $v_1v_1v_1v_1v_2v_2v_2v_2$ IV: 5-7 III: <1 II: 10-16 I: <2	7x <i>R. vitifolius</i> \times <i>R. loganobaccus</i> $v_1v_1v_1v_1v_2v_2v_2i$ IV: 0-4 III: 3+ II: 14+ I: 5-12
6x <i>R. vitifolius</i> \times <i>R. idaeus</i> (4x) $v_1v_1v_2v_2ii$ IV: <1 III: <0.5 II: 20.5 I: <0.5	6x <i>R. loganobaccus</i> $v_1v_1v_2v_2ii$ IV: 0 III: 0 II: 20.9 I: <0.5
5x <i>R. vitifolius</i> \times <i>R. idaeus</i> (2x) $v_1v_1v_2v_2i$ IV: 0-1, M 0.5 III: 0-4, M 1.6 II: 8-13, M 11.4 I: 3-7, M 5.3	5x <i>R. loganobaccus</i> \times <i>R. idaeus</i> (4x) $v_1v_1v_2v_2i$
4x <i>R. idaeus</i> iiii IV: 1-6, M 3.1 III: 0-1, M 0.6 II: 0-9, M 6.0 I: 0-5, M 1.7	4x <i>R. loganobaccus</i> \times <i>R. idaeus</i> (2x) v_1v_2ii IV: 0 III: <1 II: 7+ I: 4-9
3x <i>R. idaeus</i> iii IV: 0, M 0.0 III: 2-7, M 5.1 II: 0-5, M 1.9 I: 0-5, M 1.9	2x <i>R. idaeus</i> ii 7 ¹¹

In the same way the *R. idaeus* chromosomes form seven bivalents in the hybrid, thus confirming the conclusions based on the cytological behaviour of the Hailshamberry that this variety is an auto-tetraploid.

VI. THE ORIGIN AND CONSTITUTION OF THE LOGANBERRY

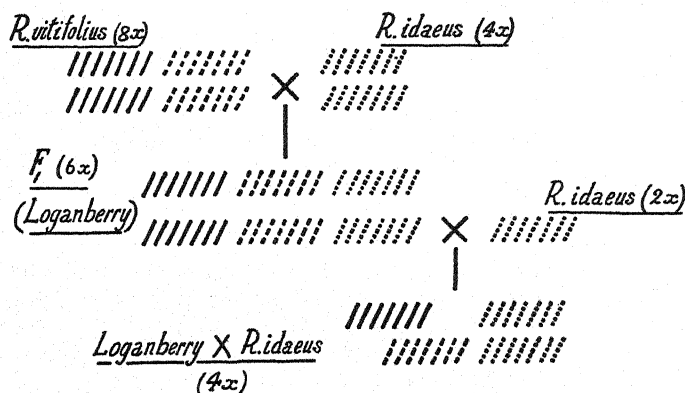
In the hybrid *R. vitifolius* \times *R. idaeus* (4x) we have a plant which is strictly comparable with the loganberry if we regard the latter as having arisen by fusion of an *R. vitifolius* gamete with a diploid gamete from *R. idaeus*. The two plants should therefore compare strictly in form and behaviour.

Crane (1940b) has shown that they are essentially the same morpho-

logically and genetically. The present data (Table IV) show that they also agree in cytological behaviour.

The chromosomal relationships may be represented diagrammatically as in Text-fig. 12. The female gamete from *R. vitifolius* contains two different pairs of identical sets of chromosomes ($\mathbf{v_1v_1v_2v_2}$) and the male gamete from the tetraploid *R. idaeus* will contain one pair of identical sets (\mathbf{ii}). The F_1 hybrid between these two species is an allo-hexaploid in which pairing is almost completely by bivalent formation.

It is true that occasional quadrivalents are formed in this hybrid, whereas they have not been observed in the loganberry. But this slight difference in behaviour may be due to either or both of two causes:



Text-fig. 12. Diagram illustrating the probable chromosome constitution of the loganberry and its behaviour when backcrossed to a diploid raspberry.

(1) The precise parents of the loganberry may have been genetically different in some minor respects from those used in the above cross.

(2) The loganberry when it first originated may have behaved like the above hybrid, but have differentiated in course of time towards a more strict allo-polyploidy. As Crane & Darlington (1927) point out, the loganberry is not a clone and sexual reproduction offers the opportunity for such differentiation.

We may thus consider the chromosome constitution of the loganberry to be $\mathbf{v_1v_1v_2v_2ii}$. The differentiation between the $\mathbf{v_1}$ and $\mathbf{v_2}$ chromosome sets may be further shown by the behaviour of the tetraploid hybrid loganberry \times *R. idaeus* (2x). This hybrid will be of the constitution $\mathbf{v_1v_2ii}$ (Text-fig. 12). Since the \mathbf{ii} chromosomes are always expected to form seven bivalents, the failure of pairing observed in the hybrid (Table IV) must be due to differences between $\mathbf{v_1}$ and $\mathbf{v_2}$ chromosome sets.

If we substitute the chromosomes of *R. neglectus* ($2x$) or *R. niveus* ($2x$) in hybrids with the loganberry, meiotic instability is further increased (Text-fig. 11). This shows that non-pairing in the loganberry \times *R. idaeus* hybrid is due to non-autosyndesis among the *vitifolius* chromosomes (v_1, v_2) and not due to non-pairing of the outside *idaeus* with the inside *idaeus* chromosomes (Text-fig. 12).

In other words, although the twenty-eight gametic chromosomes of *R. vitifolius* in the loganberry can pair completely among themselves, the product of this pairing (fourteen chromosomes) in the gamete of the loganberry cannot do so owing to the differentiation of v_1 and v_2 chromosome sets.

VII. THE RELATIONSHIP BETWEEN *R. VITIFOLIUS* AND *R. IDAEUS* CHROMOSOMES

In the hybrid *R. vitifolius* \times *R. idaeus* ($2x$), ($v_1v_1v_2v_2i$) we saw that *R. idaeus* chromosomes could pair freely with those of *R. vitifolius*, since in only three out of the fourteen nuclei do we find seven univalents and their number may be as low as three. In an auto-triploid *R. idaeus*, where the conditions of chromosome pairing are more favourable than in the above hybrid, we find a range of 0-5 univalents with an average of two per nucleus (Tables I, IV). This behaviour suggests that the i set of chromosomes is closely related to either the v_1 or the v_2 set.

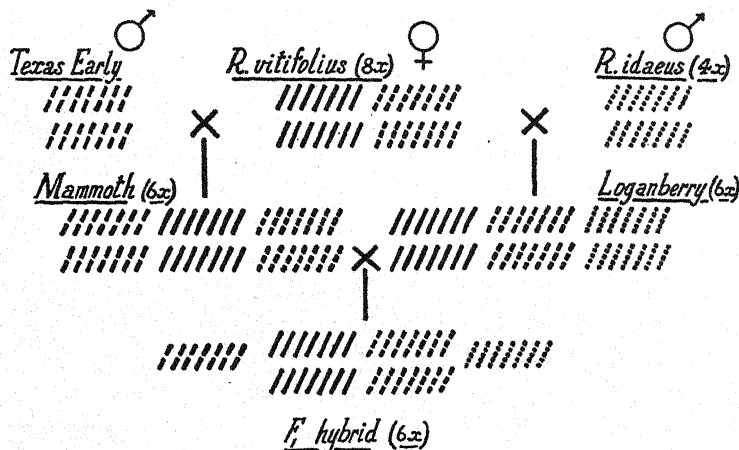
Support for this conclusion is obtained from the behaviour of *R. loganobaccus* \times *R. idaeus* ($2x$) (v_1v_2ii) where trivalents are formed occasionally (Table IV). The prevalence of quadrivalents in the hybrid *R. loganobaccus* \times *R. vitifolius*, which is of the constitution $v_1v_1v_1v_2v_2v_2i$, is evidence in the same direction. Cytology indicates, therefore, that *R. vitifolius* contains some chromosomes which are closely related to corresponding chromosomes in *R. idaeus*.

In this connexion it is of interest to note that morphologically the Rubi in the subgenus *Eubatus* section *Vitifolii* are closer to *R. idaeus* than are the true blackberries, the Fruticosi.

VIII. CHROMOSOME RELATIONSHIPS IN OTHER LOGANBERRY HYBRIDS

Darrow & Longley (1933) in their paper on the cytology and breeding of the loganberry and related blackberries do not favour the hybrid origin hypothesis. Their observations, however, when viewed in the light of the present cytological results, appear to confirm rather than to oppose the hybrid-origin hypothesis.

The "mammoth" blackberry was produced in Judge Logan's garden under similar circumstances to the loganberry. It was considered that they both arose from the same female parental species, *R. vitifolius*. A plant of this species grew near the Texas Early Blackberry and Judge Logan concluded that just as the raspberry was the male parent of the loganberry, so the Texas Early Blackberry was the male parent of the mammoth. The mammoth, like the loganberry, is a hexaploid. These forms have been crossed together by Logan and by Darrow & Longley; the hybrids proved to be sterile. The latter examined the hybrids cytologically and found that the chromosomes "were frequently unpaired in the early meiotic phases, and the irregularities in chromosome distribu-



Text-fig. 13. Diagram comparing the probable chromosome constitution of the loganberry and the mammoth berry, and the behaviour of the F_1 hybrid.

tion of later phases indicated the presence of incompatible chromosome sets".

Text-fig. 13 illustrates the chromosomal constitution of the loganberry on the basis of the present data and of the "mammoth berry" in accordance with Logan's conclusion. The mammoth berry would, from its supposed origin, differ from the loganberry in having two blackberry sets instead of two raspberry sets of chromosomes. The hybrid will therefore contain one set of blackberry and one set of raspberry chromosomes in addition to the twenty-eight chromosomes derived from *R. vitifolius*. We know that the twenty-eight *R. vitifolius* chromosomes will pair completely by auto-syndesis, so that from the point of view of chromosome association, we need only consider the seven blackberry and seven raspberry chromosomes. How far blackberry and raspberry chromosomes are

differentiated from each other is not precisely known, since no diploid hybrids between these types are available and numerous attempts to produce them artificially at Merton have entirely failed. Hybrids have only been obtained where there is chromosome duplication on one or both sides, in which cases pairing at meiosis is by auto-syndesis. As Crane (1940 *a*) points out, the failure to produce hybrids by union of haploid gametes evidently suggests some degree of differentiation between the chromosomes of the two types.

The pairing difficulties and consequent sterility observed by Darrow & Longley in the logan-mammoth hybrids appear to be due to the suggested differentiation between the seven blackberry and seven raspberry chromosomes.

Hybrids of the loganberry with two hexaploid types, the youngberry and Lucretius, were also studied cytologically by Darrow & Longley. But as the origin of these forms is even more obscure than that of the loganberry, the significance of cytological observations on their hybrids with the loganberry can only be speculative.

In the cross loganberry (♀) with youngberry (♂), however, a triploid plant was discovered. This is evidently a haplo-polyploid loganberry produced by haploid parthenogenesis. The authors describe the formation of as many as ten bivalents by the twenty-one chromosomes, but since they have recorded similar configurations in triploid *R. idaeus*, where I have never found more than seven associations (Tables I, IV), I am inclined to think that they have failed to distinguish between trivalents, bivalents and univalents in these cases. However it seems clear that a considerable degree of chromosome pairing does occur and this may be regarded as evidence in support of the suggestion that the *i* chromosome set in the gamete of the loganberry is closely related to either the v_1 or v_2 set.

IX. SUPPOSED OBJECTIONS TO THE HYBRID ORIGIN OF THE LOGANBERRY

It now remains to consider the objections that have been raised against the hybrid origin of the loganberry in the light of the present results. Perhaps the main objection is concerned with the fact that the loganberry behaves like a pure species. Darrow & Longley (1933) state that: "The chromosomes of the Logan show irregularities during meiosis too infrequently to support the view that they are made up of two sets from *R. ursinus*¹ and a red raspberry." This objection ignores the fifteen

¹ As shown by Crane (1940*b*), the species *R. ursinus* and *R. vitifolius* are very closely allied if not identical.

examples of complete auto-syndesis listed by Darlington (1932, Table XXVIII, p. 190). The production of a fertile hexaploid with regular meiosis, by crossing the auto allo-octoploid *R. vitifolius* ($\mathbf{v_1v_1v_2v_2}$) with an auto-tetraploid *R. idaeus* (ii) is merely a further example to add to this list. Another objection, that blackberry-raspberry hybrids are mostly sterile, is explained in the same way. If the two species have the correct chromosome constitution the hybrid will be fertile. The veitchberry, which contains two blackberry and two raspberry sets of chromosomes, is an example of such a fertile hybrid in which chromosome pairing is auto-syndetic.

The fertility of loganberry-blackberry crosses as opposed to the sterility of loganberry-raspberry hybrids has also been advanced as an objection to the hybrid origin of the loganberry (Darrow, 1937). This objection, however, ignores a whole series of well-established cytological facts. Fertility in a cross between two forms of *Rubus* may in certain rare instances be taken as an indication of the relationship of the parents. But it also depends on:

- (1) Whether the two parents are diploid or polyploid.
- (2) Whether the cross is balanced or unbalanced.
- (3) Whether auto-syndesis occurs in the complement of one or other of the parents or in those of both.
- (4) Whether non-sexual processes of reproduction are concerned (cf. Crane, 1940*a*; Thomas, 1940).

X. SUMMARY

1. The Hailshamberry, *Rubus idaeus* ($4x$), is found to be an auto-tetraploid (iiii).

2. *R. vitifolius* is an octoploid ($2n=56$) which on the basis of its behaviour in hybrids with diploid and tetraploid raspberries is shown to be a doubled allotetraploid, or auto-allopolyploid 2 ($\mathbf{v_1v_1v_2v_2}$) producing gametes of the constitution $\mathbf{v_1v_1v_2v_2}$.

3. When these two forms are crossed a fertile hexaploid, in which chromosome pairing is almost wholly by bivalent formation, is produced. There is thus complete auto-syndesis among the gametic chromosomes of the parental forms.

4. This hybrid agrees in cytological behaviour with the loganberry, and its origin is taken as reconstructing the history of the loganberry.

5. The loganberry therefore has the chromosome constitution $\mathbf{v_1v_1v_2v_2ii}$, and since pairing is by auto-syndesis the gametes produced will all be of the constitution $\mathbf{v_1v_2i}$.

6. The differentiation between the v_1 and v_2 chromosome sets derived from *R. vitifolius* is shown by the failure of pairing in the tetraploid hybrid loganberry \times *R. idaeus* (2x) (v_1v_2ii).

7. If we substitute chromosomes of *R. niveus* (2x) or *R. neglectus* (2x) for *R. idaeus* in hybrids with the loganberry, meiotic instability is further increased. This shows that non-pairing in the loganberry \times *R. idaeus* hybrid is due to non-auto-syndesis among *vitifolius* chromosomes (v_1v_2), and not to non-pairing among *idaeus* chromosomes, i.e. those already in the loganberry gamete and those brought in from outside.

8. Evidence suggests that the *i* set of chromosomes in the gamete of the loganberry is closely related to either the v_1 or v_2 set derived from *R. vitifolius*. Trivalents are formed in the hybrid, loganberry \times *R. idaeus* 2x (v_1v_2ii) and the number of univalents in the hybrid *R. vitifolius* \times *R. idaeus* (2x) ($v_1v_1v_2v_2i$) may be as low as three.

9. Support for this suggestion is obtained from the close association observed by Darrow & Longley in a haplopolyploid parthenogenetic loganberry (v_1v_2i).

10. In short, the chromosome behaviour of the loganberry, of its supposed parents and of its crosses with them is consistent with its reputed hybrid origin. This is equally true of the evidence which has been used to oppose this view.

REFERENCES

- CRANE, M. B. (1940 a). "Reproductive versatility in *Rubus*. I." *J. Genet.* **40**, 109.
 — (1940 b). "The origin of new forms in *Rubus*. II." *J. Genet.* **40**, 129.
 CRANE, M. B. & DARLINGTON, C. D. (1927). "The origin of new forms in *Rubus*. I." *Genetica*, **9**, 241-78.
 DARLINGTON, C. D. (1932). *Recent Advances in Genetics*, 1st ed. London: Churchill.
 DARROW, G. M. (1937). "Blackberry and raspberry improvement." *Yearb. U.S. Dep. Agric.* pp. 496-533.
 DARROW, G. M. & LONGLEY, A. E. (1933). "Cytology and breeding of *Rubus macro-petalus*, the logan- and related blackberries." *J. agric. Res.* **47**, 315-30.
 LEWIS, D. (1940). "The relationship between polyploidy and fruiting habit in the raspberry." *Proc. 7th int. Congr. Genet., Edinburgh*.
 LONGLEY, A. E. & DARROW, G. M. (1924). "Cytological studies in diploid and polyploid forms in raspberries." *J. agric. Res.* **27**, 737-48.
 THOMAS P. T. (1940). "Reproductive versatility in *Rubus*. II." *J. Genet.* **40**, 119.

EXPLANATION OF PLATE VI

- Fig. 1. Diakinesis in *R. vitifolius* showing quadrivalents. ($\times 2000$.)
Fig. 2. Somatic chromosomes in the hybrid *R. vitifolius* \times *R. idaeus* ($4x$) ($2n=42$). ($\times 2000$.)
Fig. 3. Oblique polar view of metaphase I in loganberry \times *R. vitifolius* ($2n=49$). ($\times 1000$.)
Fig. 4. Metaphase II in the same hybrid, showing the orientation of univalents. Note the non-random distribution of chromosomes in the lower cell in which there are twenty-four chromosomes at one pole and the remainder divided into two groups at the other end. ($\times 1000$.)
Fig. 5. Metaphase I in the loganberry showing univalent. ($\times 2000$.)
Fig. 6. Pollen germination in the "synthetic loganberry", *R. vitifolius* \times *R. idaeus* ($4x$).

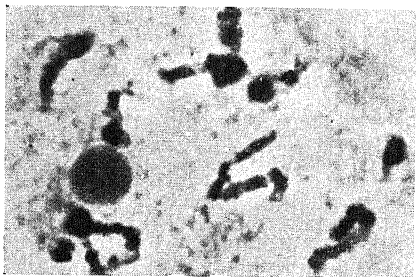


Fig. 1.

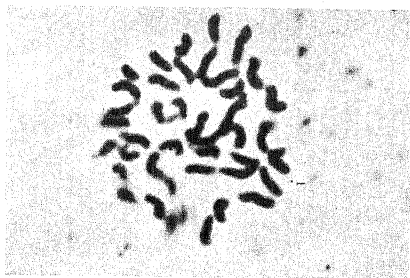


Fig. 2.

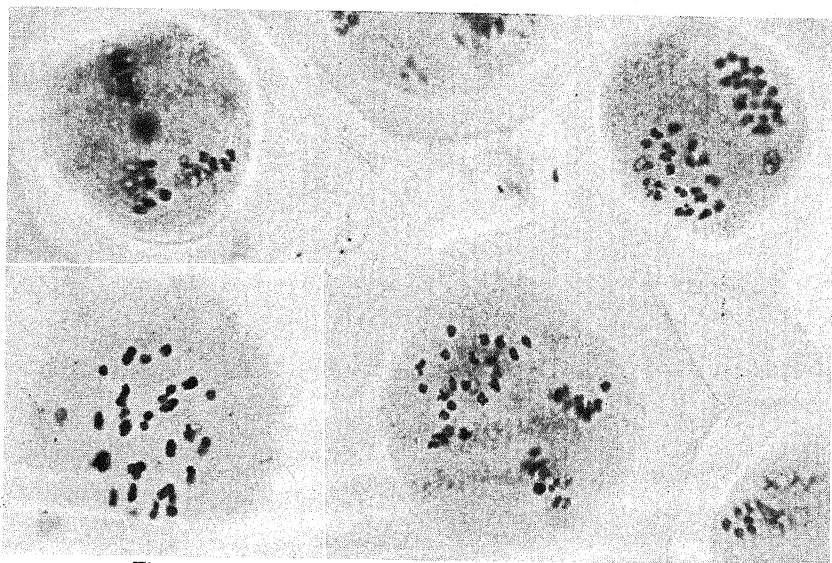


Fig. 3.

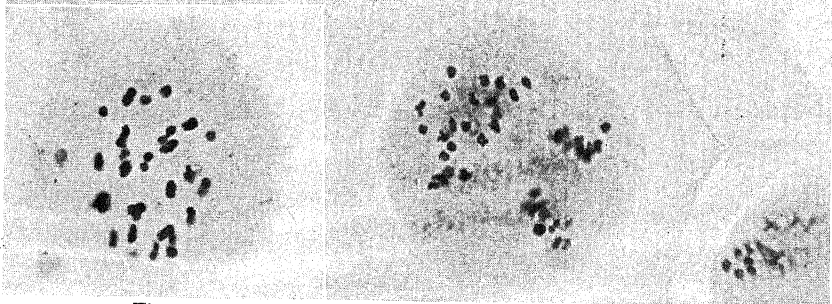


Fig. 4.

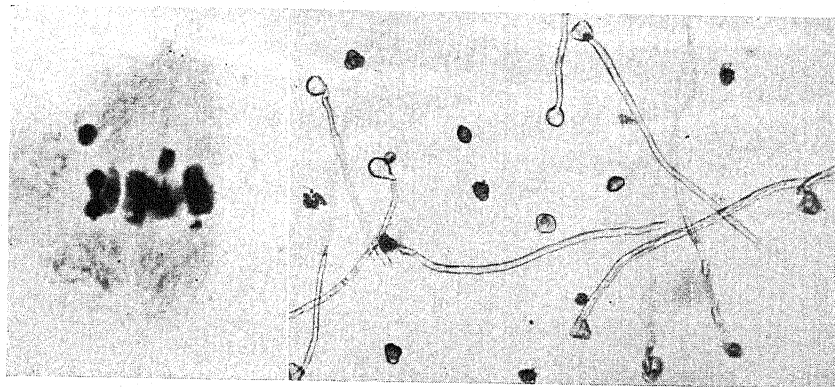


Fig. 5.

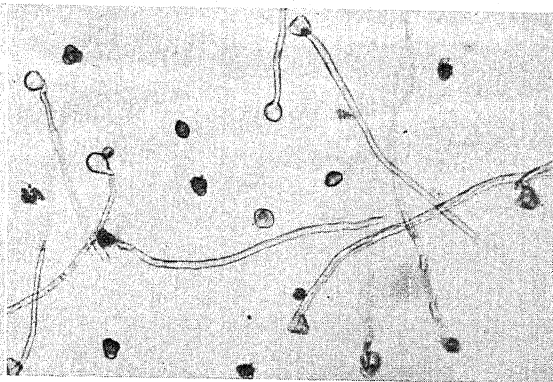
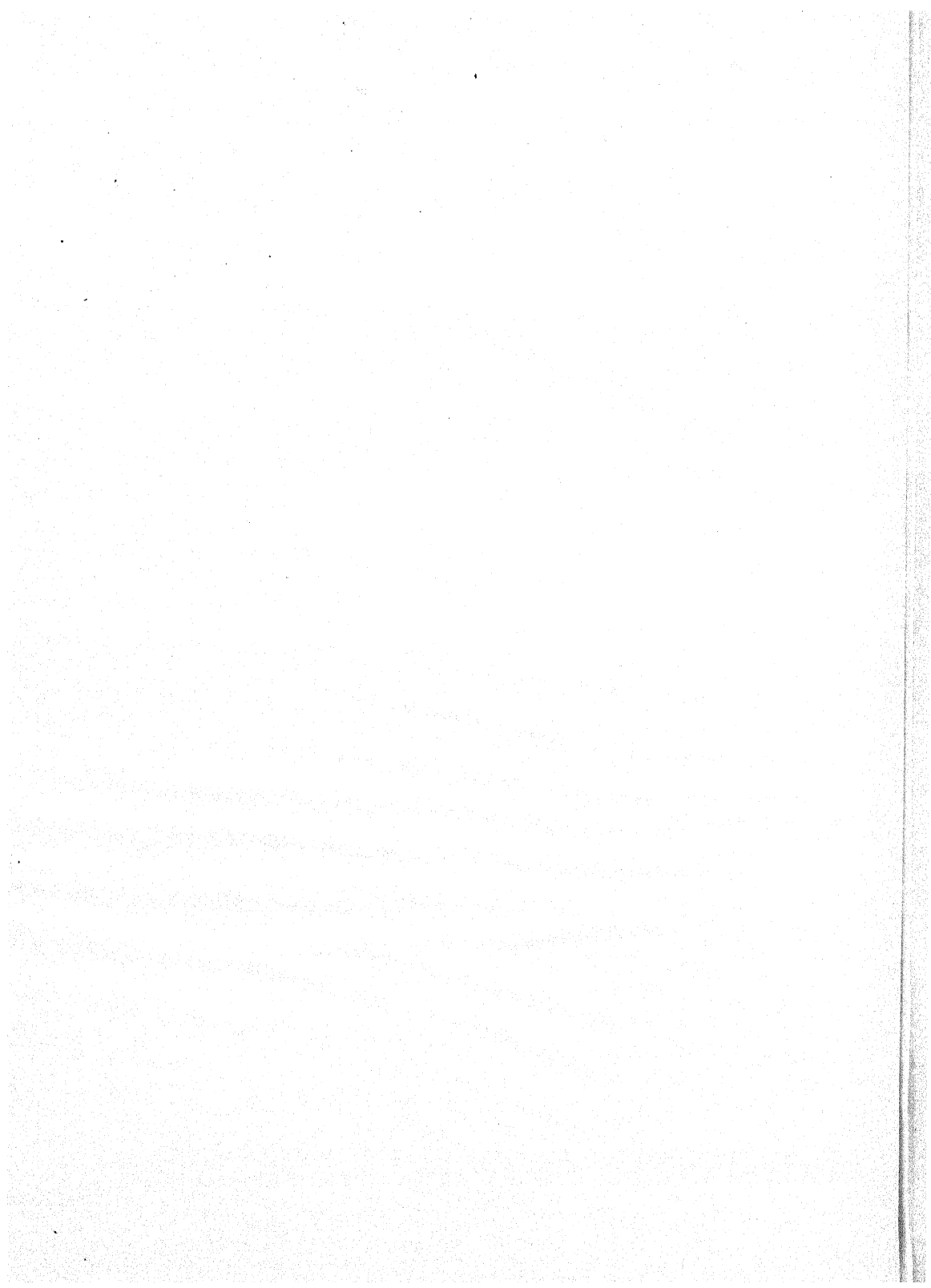


Fig. 6.



HEREDITARY AND ENVIRONMENTAL FACTORS IN THE ORIGIN OF DIFFERENT CANCERS¹

By N. DOBROVOLSKAIA-ZAVADSKAIA

Institute of Radium, University of Paris

(With Seven Text-figures)

THE enormous efforts made by geneticists in the investigation of hereditary transmission of cancer susceptibility in mice have resulted in the accumulation of a substantial body of evidence in favour of such a transmission.

It is a well-established fact that there are strains in which mammary cancer appears in a high proportion of females, and there are others in which this malignancy type is more or less completely lacking. This difference can hardly be accounted for by environmental conditions which, for mice colonies, are in general similar for all strains. But frequently the behaviour of a strain, as regards cancer, is determined by the origin of this strain from a cancerous or non-cancerous mother. This may be illustrated by the following observation.

A pregnant cancerous female was brought to the laboratory a few years ago by a dealer who complained that his mice stock had been devastated by frequent occurrences of tumours. This female gave birth to three young of which one male and one female attained adult age. Being crossed brother to sister, these two animals bred many times and died without cancer at an advanced age, the male when 16 months old and the female at the age of 31 months. The progeny of this couple (strain XLIV) recorded here consists of 75 males and 88 females, of which only two females developed a mammary cancer (2.3 %).

This result was disappointing, as we expected to establish a high cancer strain. However, it may be easily explained by the fact that the real female ancestor of this strain was not the cancerous female brought pregnant to the laboratory, but her daughter which was born from an unknown father, and which died at advanced age without cancer.

The incidence of mammary cancers in various strains is very different; for example, in our mice colony, it fluctuates, according to the strain, from 0 to 75 %. In strains submitted to a prolonged inbreeding (dilute-brown strain of Dr Little, "A" strain of Dr Strong, etc.), this incidence is

¹ This paper was presented to the Seventh International Congress of Genetics, August 1939, Edinburgh.

considered as more or less constant. We tried to verify this constancy in our high cancer strain R III.

If the incidence of mammary cancers does not change with the passage of time, any sample of animals born in a certain period should present the same percentage of cancers, as does the total number of animals belonging to this strain. According to this, we divided the 8-year period of observation of strain R III into six separate periods and the percentage rate of mammary cancers was calculated for each period. Only those females (465 ♀♀ in all) which survived at least 5 months were taken into consideration. We plotted the time periods as abscissae and the percentage rate of tumours as ordinates. The graphic representation of the results obtained shows a curve with two peaks (periods I and V) and

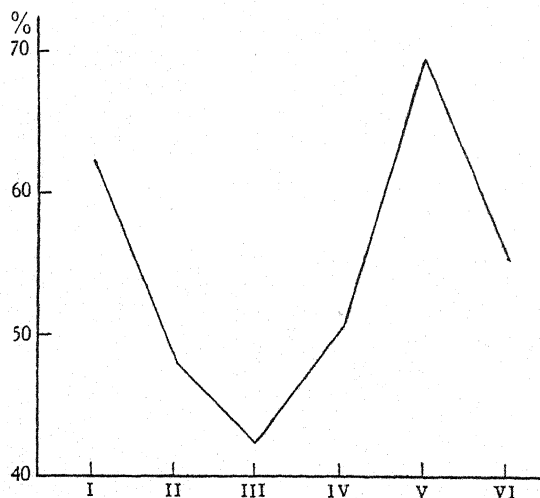


Fig. 1. Strain R III, variation of the percentage rate of spontaneous tumours (mammary adenocarcinoma) during 8 years of observation divided in six periods (abscissae).

fluctuation of the percentage rate of tumours from 42.3 (period III) to 69 % (period V). The difference between two extremes is 26.7 %, which is significant and must be taken into account (see Fig. 1).

The average percentage rate of mammary cancers in strain R III is 53.5, which approaches the simple Mendelian proportion (1 : 1) for a backcross. Such a high proportion of malignancy (in a few extinct strains it even reached 75 %) was obtained only for mammary adeno-carcinoma.

The proportions of spontaneous sarcomas and squamous cell epitheliomas which occurred in our stock were too small to be considered as depending on a simple Mendelian mechanism. However, the hereditary background of these histological forms cannot be excluded. We had a

strain (strain IV) derived from a female which died of a molluscoid tumour of Borrel-Haaland. Her son, the only male parent of the whole second generation, died of sarcoma. In this strain, there were observed five sarcomas (4.1 %) and two squamous cell epitheliomas (1.7 %) in 121 males and seventeen sarcomas (14.3 %) and four squamous cell epitheliomas (3.4 %) in 119 females, i.e. many more than in any other of our strains, but not enough for monofactorial heredity. We did not succeed in perpetuating this condition in subsequent generations.

Maud Slye also mentioned (1931, 1937) strains with high incidence of sarcoma, namely, 28.5 % in one strain and 32.9 % in another one.

Lymphadenoma, a kind of pseudoleukemia, gave as the highest proportions 22.4 % of malignancy in males and 18.4 % in females. But this was only in strain III which does not exist any more. These proportions are lower than those obtained for similar neoplastic conditions by Mercier (1937) (50-70 %) and MacDowell (1937) (90 %).

Consequently, only *the mammary cancer* has given us a sufficient proportion of tumours to serve as a test for experimentation upon the *relative part of hereditary and environmental factors in the pathogenesis of various cancers*.

The environmental influences were studied on *five non-cancerous strains*, that is to say, strains free from spontaneous mammary cancer, and on many cancerous strains, especially on our high cancer strain R III. Tar, 1 : 2 : 5 : 6-dibenzanthracene, and radon, used as extraneous pathogenic agents, answered our purpose most satisfactorily (Dobrovolskaia-Zavadskaia & Adamova, 1938, 1939).

Out of a total of 1547 animals (♂ 772 and ♀ 775) belonging to *non-cancerous strains*, 692 were kept as controls, 103 males were treated with tar, 445 animals (♂ 187 and ♀ 258) with dibenzanthracene, 198 (♂ 92 and ♀ 106) with radon and 111 (♂ 82 and ♀ 29) we infested with *Sp. morsus muris*. Sixty of the infested animals also had a tube of radon introduced under the skin of the left groin.

The following percentage rates of tumours were obtained: 59.2 % (+10 % of precancerous lesions) with tar, 19.3 % in males and 26 % in females with dibenzanthracene; 8.8 % in males and 5.7 % in females with radon. Among the infested animals, only one developed a sarcoma surrounding the tube of radon, and seven animals died with more or less pronounced lymphatic hyperplasia (7.2 % on the whole).

In respect to histological types, the local reaction was nearly the same in all these strains, namely, squamous cell epithelioma, very rarely a sarcoma, with tar; sarcomas of various structure, very rarely a squamous

cell epithelioma, with dibenzanthracene; sarcoma and squamous cell epithelioma with radon, and practically no mammary adenocarcinoma at all.

Two of these strains, XVII *nc* and XXXIX (our branch of Dr Strong's C.B.A. strain) were used by Dr Lacassagne for folliculine injections. Although the folliculine proved to be a very powerful stimulator for cancerization of the mammary gland, not one case of mammary cancer was obtained either in males, or in females.

Tar, 1 : 2 : 5 : 6-dibenzanthracene, and radon were also used in the animals belonging to our cancerous strains. These animals reacted in similar way to the animals of non-cancerous strains, with the exception that in females there appeared, in addition to sarcomas and squamous cell epitheliomas, some mammary cancers on the point of carcinogenic application, and much more frequently all over the body.

In order to investigate whether these local mammary cancers were of genetical or environmental origin, we proceeded to a detailed study of the topographical distribution of tumours in females of strain R III (Dobrovolskaia-Zavadskaia & Adamova, 1939). In 465 control females there were 249 cancerous animals with a total of 384 tumours. These tumours were scattered all over the body of the animals; we determined their percentage in different anatomical areas. Two of such areas were concerned in our experiments: (1) in experiments with dibenzanthracene, the left axilla and the surrounding parts of the scapular girdle, we found there 17.7 % of all the tumours and (2) in experiments with radon, the left groin and the surrounding parts of the pelvic girdle, 15.9 % of tumours were located in this area.

Out of sixty-two mammary cancers developed by the females treated with dibenzanthracene, only ten (16.1 %) were located at the point of carcinogenic application. Out of fifty-seven mammary cancers obtained in females treated with radon, only four (7 %) were located near the radon tube at the left pelvic girdle. The number of mammary cancers then was not increased at the points of carcinogenic application. It was slightly diminished with dibenzanthracene, 16.1 % instead of 17.7 % in control animals, and it presented with radon less than a half (7 % instead of 15.9 %) of what was determined for non-treated animals.

Consequently, the environmental factors proved to be unable to produce any glandular cancer not only in non-cancerous strains, but also in a high cancer strain R III. Therefore, mammary cancers which appeared in treated areas were not induced but were spontaneous tumours. They have arisen in these areas according to the genetical predisposition of their carriers.

The existence of such local predisposition is also evidenced by the fact that the great majority of mammary tumours (84 % in the dibenzanthracene series and 93 % in the radon series) were developed not on the point of environmental carcinogenic activity, but in their habitual locations (see Figs. 2, 5).

The mammary cancers developed on the point of carcinogenic application very often presented a tendency to squamous cell metaplasia of glandular epithelium and to sarcomatization of the stroma. It may be



Fig. 2. ♀ 38010 XL treated with dibenzanthracene. No tumours at the point of injection, left axilla. Spontaneous mammary cancer in the left groin.

supposed that missing mammary tumours in the radon series were also replaced by sarcomas or squamous cell epitheliomas. This shows that the two latter types of malignancy may be actually produced by environmental factors. As a matter of fact, none of the tested strains proved to be completely resistant against external carcinogenic influences, and many sarcomas and squamous cell epitheliomas were obtained in cancerous as well as in non-cancerous strains.

This brings us back to the once expressed hypothesis (Dobrovolskaia-Zavadskaia, 1933) that there exists a factor of general neoplastic pre-



Fig. 3. ♀ 38909 XL. Nothing at the point of injection of dibenzanthracene, spontaneous mammary gland tumour in the right groin.



Fig. 4. ♀ 25108 R III, treated with radon, left groin. No tumour on the scar from radon tube, mammary adenocarcinoma of the nape.

disposition, we called it *no* (neoplasm). It seems to be widely spread in mice, and is perhaps responsible for the frequently observed appearance of cancerous animals in the first hybrid generation after crossing representatives of cancer and non-cancer strains. The assumption that spontaneous mammary cancer is dominant is opposed by the fact that it may be transmitted by non-cancerous animals.

The general factor of malignancy *no* is probably present in all non-cancerous strains, only it does not operate for want of a modifier for



Fig. 5. ♀ 38735 XL treated with radon, left groin. No tumour at the point of radon tube, mammary gland cancer in the right axilla.

“tissular” or organic localization. This modifier may be dominant since it operates in the above-mentioned hybrids in one dose. Such a mechanism may be operating for tumours in *Nicotiana* hybrids (Kostoff, 1930).

The modifier for mammary cancer seems to be closely linked with *no* and thus determines the monohybrid attitude of this cancer. The proportions which we obtained in the above-mentioned strain IV for sarcomas and squamous cell epitheliomas resulted rather from a free segregation.

The experiments with carcinogenic environmental factors have

yielded evidence in favour of the possibility of replacing this morphogenic modifier by an external agent. The comparative data obtained in non-cancerous and cancerous strains suggested that external agents may be operating so only for sarcomas and squamous cell epitheliomas but not for the mammary gland cancer. Further investigations are needed to elucidate whether or not the other glandular cancers behave in the same way.

Previous observations (Dobrovolskaia-Zavadskaja, 1934) of the hereditary transmission of mammary cancer at a specially rare location in our strains, i.e. the nape of the neck, brought us to the hypothesis of localizing modifiers. The existence of such modifiers did find its confirmation *in the extreme perseverance with which mammary cancers have kept on appearing at their usual sites outside of the zone of carcinogenic application*. On the other hand, sarcomas and squamous cell epitheliomas, at least as induced tumours, appeared only at the points of carcinogenic irritation.

The full genetic verification of the suggested interpretation is extremely difficult. The genetic constitution in the matter of cancer becomes in general clear only in advanced age when the animal is in most cases no longer capable of breeding. Still, we had a chance at genetic verification of a sarcoma developed on the point of a chronic inflammatory irritation, a circumscribed peritonitis, in a female two years old (Fig. 6). This female had nine generations without cancer before her, and she left numerous progeny (seventy-five offspring) after crosses with her brother, her son and her grandson. This mouse was the only female ancestor of our selected strain XVII *nc*, and not one case of spontaneous sarcoma was ever found in non-treated animals of this strain. This observation confirms our hypothesis that an environmental carcinogenic factor may play the part of the morphogenic modifier in the origin of a sarcoma.

The manifestation of the factor *no* in the strain XVII *nc* may be seen in many sarcomas and squamous cell epitheliomas provoked by carcinogenic agents. The genic background of this manifestation has been betrayed by the disclosure in treated animals of many resistant individuals which lived sometimes longer than those developing tumours and which died without cancer.

We come thus to the general conclusion that *heredity controls the pathogenesis of mammary cancer in mice whereas the environment dominates the origin of sarcomas and squamous cell epitheliomas*. The latter part of this conclusion opens the way to some optimistic prospects of preventing, at least some cancers, by an adequate change of environmental conditions. But what can be done with hereditary types of malignancy?

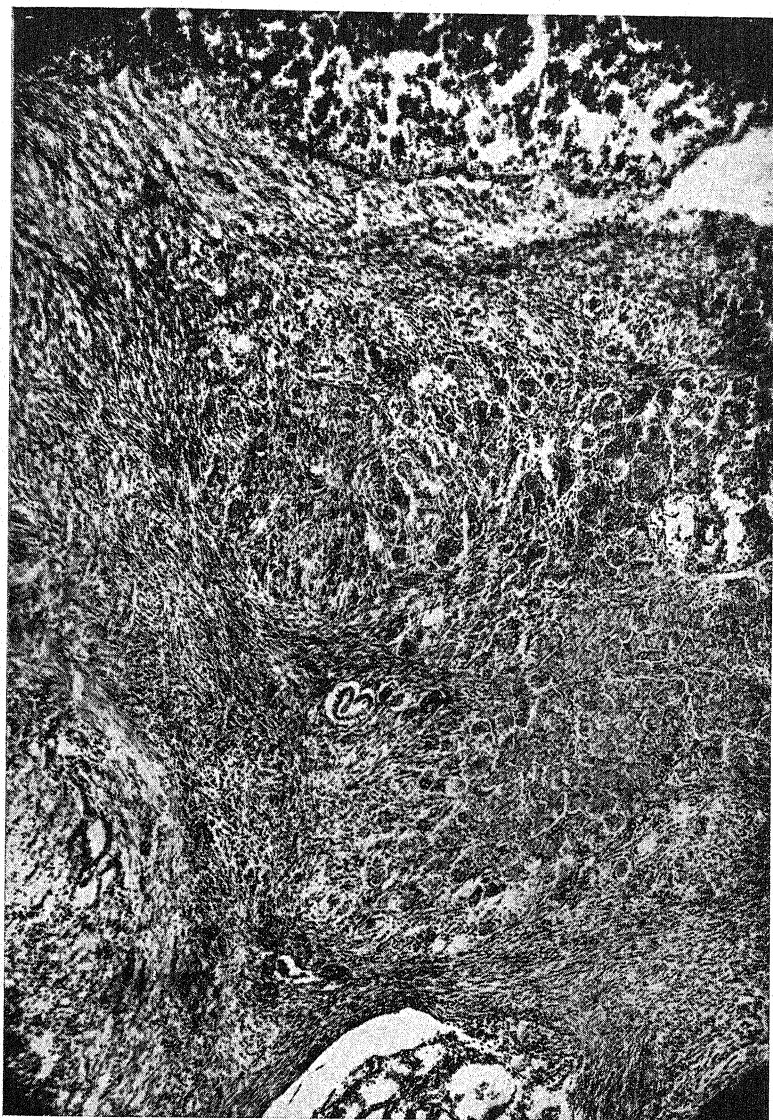


Fig. 6. ♀ 33977 XVII *nc*. Sarcoma developed at the point of a chronic intraperitoneal inflammation. The tumour involves the spleen (upper part) and the uterine tromp (lower part) and infiltrates the pancreas (in the middle of the preparation).

The animals descending from the strain R III were used by Lacassagne (1932) and by Cramer & Horning (1937) for the experiments with oestrogenic substances. A very surprising feature is that these experiments have given practically 100 % of mammary cancers both in males and in females. However, as we mentioned before, the same treatment failed to produce any such cancer in animals of non-cancerous strains XVII *nc* and XXXIX (Lacassagne, 1938). This statement emphasizes the importance of constitutional factors in the origin of mammary cancer. At the same time, it makes conclusive that all animals of the strain R III are susceptible to this cancer, that is to say that the strain R III is obviously homozygous for cancer. Why then do not all animals living long enough develop it spontaneously?

The underlying mechanism by which genic control is brought about is as yet unknown. The term "inhibition" has been used in the mendelian literature to designate the general situation in which one genetic factor prevents another non-allelomorphic factor from showing its effects. This inhibition, as stated in *Drosophila*, may be due to the presence of a sectional duplication carrying the wild type allelomorph of the gene "suppressed" the term now in use; or to a gene "specific suppressor" which gives a positive reversal of the effect of another non-allelomorphic gene, so that the double mutant type appears like the unmutated wild type (Bridges, 1919, 1923, 1932; Stern, 1929).

In the case of cancer, it is extremely difficult to establish a sufficient basis of facts for an analysis of such "normal overlaps" in terms of genes. Let us dwell a little on what is called "internal environment", by which we mean "genic environment" (Lebedeff, 1935) on the one hand, and "somatic environment" on the other.

The genic environment is certainly extremely various in our strains. Side by side with traits which seem to have nothing to do with cancer—coat colour, taillessness, waltzing, etc.—there are some pathological conditions, other than cancer, which are frequently encountered in some strains, and are practically lacking in others. To illustrate this, we can refer to a modification of the reticular zone of the adrenal gland described by Cramer & Horning (1937) under the name of "brown degeneration".

This degeneration is extremely frequent in strain R III (Fig. 7), nearly 100 % of the cancerous females develop it, and it is practically lacking in five non-cancerous strains. However, this degeneration is not absolutely necessary for cancer development. We have a cancerous strain (strain XIX) in which about 90 % of the cancerous females proved to be free of it (Dobrovolskaia-Zavadskaia & Pezzini, 1939). The kidney also pre-

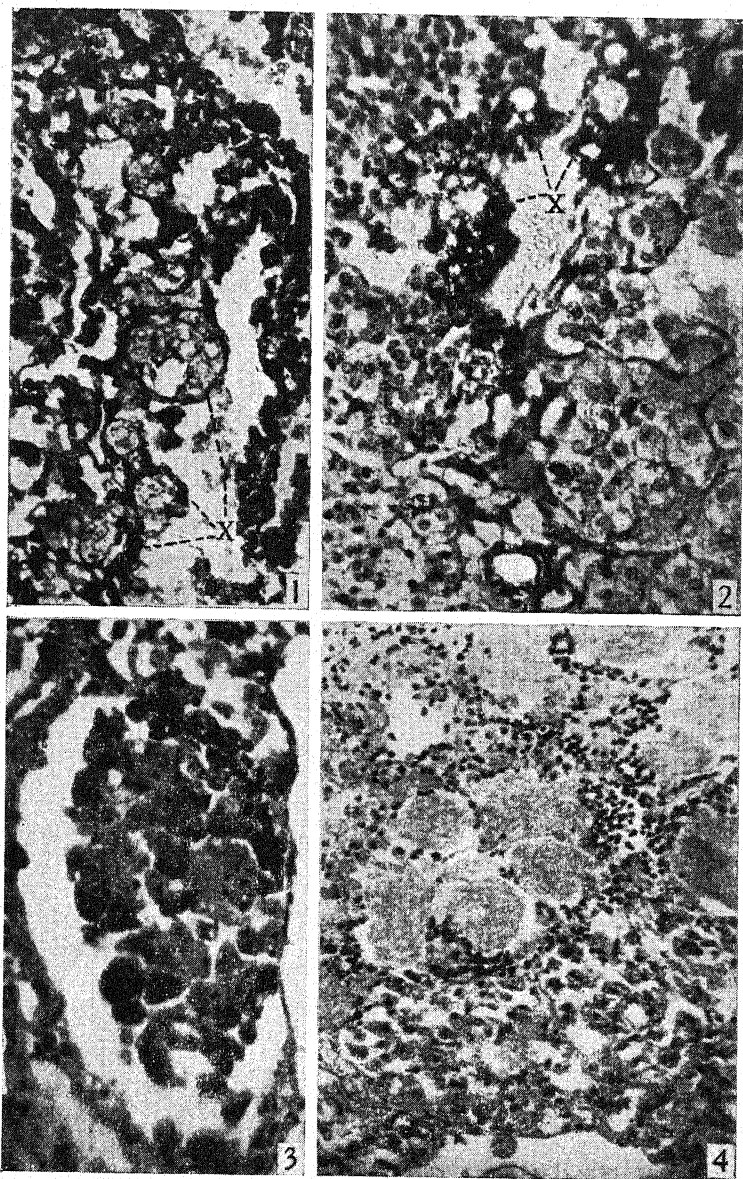


Fig. 7. Different aspects of degeneration in the reticular zone of adrenal glands: (1) vacuolated blocks (X), (2) vacuolated and pigmented blocks and isolated cells (X), (3) "brown degeneration", cells with coagulated cytoplasm and pycnotic nuclei in the way of fusion, (4) completely degenerated blocks, appeal of leucocytes.

sented some constitutional traits—hyalinization, cyst formation—peculiar only to some strains and completely absent in others. Lesions of the liver and of the spleen are frequent in cancerous mice, and it is possible that some of these lesions, and especially functional peculiarities of endocrine glands, also depend on hereditary constitution. We know very little about all this. However, the above-mentioned observations show that the genic environment of the cancer gene is different in different individuals.

The somatic aspect of the internal environment has become evident from the experiments with oestrogenic substances. Folliculine injections started at an early age change the medium of the growing organism in such a way that every animal, male as well as female, genetically susceptible to mammary cancer, actually develops it. Thus, normal overlaps practically disappear.

There is evidence now at hand, i.e. Bittner's experiments with milk, which if proved will open a possibility of changing the somatic environment in the opposite sense. An offspring hereditarily predisposed to cancer may perhaps be transformed in a normal overlap by substituting, immediately after birth, the cancer-susceptible mother by a cancer-resistant foster-mother. Will it not be the way to prevent at least cancer of the breast?

SUMMARY

1. Animals belonging to eighteen strains of established genetic constitution were used for investigation of environmental influences as represented by tar, 1 : 2 : 5 : 6-dibenzanthracene, and radon.

2. Not one strain proved to be completely resistant, but resistant individuals were observed in all strains.

3. Sarcomas and squamous cell epitheliomas appeared as local reactions in animals of all strains. This is an evidence in favour of environmental factors as a provocative cause of these tumours.

4. Mammary cancers occurred at the points of carcinogenic application in animals of cancerous strains. These tumours, according to the proportions in which they occurred, were not induced but were spontaneous tumours.

5. Many more mammary cancers appeared in animals of cancerous strains outside of the zone of carcinogenic application, i.e. in their habitual locations.

6. The importance of the hereditary factor in the origin of mammary gland cancer in mice was thus confirmed. In what degree is it true for

other glandular cancers may be elucidated by a similar investigation on adequate strains.

7. As regards sarcoma and squamous cell epithelioma, two kinds of pathogenesis may be distinguished: (a) one hereditary, as illustrated by an increased occurrence of sarcomas in strain IV the male ancestor of which died of a sarcoma, and (b) one environmental, as stated by a frequent appearance of sarcomas and squamous cell epitheliomas in treated areas in animals of all strains. A sarcoma surrounding an inflammatory focus was verified genetically and proved to be not hereditary.

8. Not all susceptible animals actually develop cancer. These "normal overlaps" practically disappear in susceptible animals treated with some oestrogenic substances; this may be explained by a change of "internal environment".

9. The early fostering of a cancer susceptible offspring by a cancer resistant foster-mother seems to be able to change this "internal environment" in the opposite sense, i.e. to transform such an offspring in a "normal overlap". This statement if confirmed may open a way of preventing mammary cancer and perhaps some other pathological conditions.

This work was carried out with the aid of grants from the International Cancer Research Foundation, President Mr William H. Donner.

REFERENCES

- BITTNER, J. (1929-30). "The experimental determination of an invisible mutation." *Mich. Acad. Sci. Arts Lett.* **11**, 349-51.
- (1939). "Breast cancer in mice." *Amer. J. Cancer*, **36**, 44-50.
- BRIDGES, C. B. (1919a). "Duplication." *Anat. Rec.* **15**, 357.
- (1919b). "Vermilion deficiency." *J. gen. Physiol.* **1**, 645-56.
- (1923). "The translocation of a section of chromosome II upon chromosome III in *Drosophila*." *Anat. Rec.* **24**, 426.
- (1932). "The suppressors of purple." *Z. indukt. Abstamm.- u. VererbLehre*, **60**, 207-18.
- CRAMER, W. & HORNING, E. S. (1937). "Adrenal changes associated with oestrin administration and mammary cancer." *J. Path. Bact.* **44**, 633-42.
- DOBROVOLSKAIA-ZAVADSKAIA, N. (1933). "Heredity of cancer susceptibility in mice." *J. Genet.* **27**, 181-98.
- (1934). "Über den Erblchkeitsfactor bei der Entstehung des Krebses." *M Schr. Krebsbekämpfung*. Heft 6, 161-8.
- (1936). "Facteur constitutionnel (héréditaire) dans certaines maladies des reins." *III Internat. Cong. compar. Pathol. Athènes*, **2**^a, 241-47.

- DOBROVOLSKAIA-ZAVADSKAIA, N. & ADAMOVA, N. (1938). "Réaction, à différents agents cancérogènes, de souris appartenant à des lignées exemptes d'adénocarcinome de la mamelle." *Bull. Cancer*, **27**, pp. 308-41.
- (1939). "Réaction, à différents agents cancérogènes, de souris appartenant à la même lignée cancéreuse (lignée R III)." *Bull. Cancer*, **28**, 76-106.
- DOBROVOLSKAIA-ZAVADSKAIA, N. & PEZZINI, Z. M. (1939). "Dégénérescence des capsules surrénales chez les souris de différentes lignées cancéreuses." *C.R. Soc. Biol., Paris*, **131**, 240-3.
- KOSTOFF, D. (1930). "Tumours and other malformations on certain *Nicotiana* hybrids." *Zbl. Bakt.* **81**, 244-60.
- LACASSAGNE, A. (1932). "Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine." *C.R. Acad. Sci., Paris*, **195**, 630-2.
- (1938). "Statistique des différents cancers constatés dans des lignées sélectionnées de souris, après action prolongée d'hormones oestrogènes." *Bull. Cancer*, **27**, 96-116.
- LEBEDEFF, G. A. (1935). "Further studies on factor interaction in *Drosophila virilis*." *Genetics*, **20**, 223-9.
- MACDOWELL, E. C. (1937). "Genetics of mouse leukemia." *Occ. publ. Amer. Ass. Adv. Sci.* no. 4, pp. 42-4.
- MERCIER, L. (1937). "Hérédité du lymphosarcome de la souris dans les croisements d'hétérozygotes pour le couple de facteurs cancer-non cancer." *C.R. Soc. Biol., Paris*, **124**, 403-4.
- MERCIER, L. & GOSSELIN, L. (1936). "Essais en vue de retarder l'apparition du cancer (lymphosarcome) dans une lignée de souris." *C.R. Soc. Biol., Paris*, **121**, 125-6.
- SCHULTZ, J. (1932). "The behaviour of vermilion-suppressor in mosaic." *Proc. nat. Acad. Sci., Wash.*, **18**, 485-6.
- SCHULTZ, J. & BRIDGES, C. B. (1932). "Methods for distinguishing between duplication and specific suppressors." *Amer. Nat.* **66**, 323-34.
- * SLYE, M. (1931). "The relation of heredity to the occurrence of spontaneous leukemia, pseudoleukemia, lymphosarcoma and allied diseases in mice." *Amer. J. Cancer*, **15**, 1361-86.
- (1937). "Heredity in the occurrence of cancer." *Ile Congrès Intern. de la lutte contre le cancer, Bruxelles*, 1936, **2**, 128-33.
- STERN, C. (1929). "Über die additive Wirkung multipler Allele." *Biol. Zentr.* **49**, 261-290.

STUDIES IN *HEBE*

II. THE SIGNIFICANCE OF MALE STERILITY IN THE GENETIC SYSTEM

By O. H. FRANKEL

Wheat Research Institute, Christchurch, New Zealand

(With Ten Text-figures)

INTRODUCTION

MALE sterility has been extensively studied by genetic, cytological and taxonomic methods. It is inherited as a simple recessive or, less often, cytoplasmically. Degeneration occurs in the course of meiotic or pollen development, and in a number of cases it is associated with irregular chromosome or spindle behaviour. Plants with non-functional male organs, found in numerous hermaphrodite species, have been termed "females", and such species have been classed as "gynodioecious". The time has arrived for a correlation of the genetic and cytological evidence with taxonomic findings. We must no longer see male sterility as an abnormality of experimental conditions or as a systematic phenomenon without reference to its origin and variability. We must regard it as an adaptive mechanism whose occurrence in nature follows from the experimental observations, which enable us to get a view of its role in the reproductive systems of plants. The present paper is a first attempt to do this.

Male sterility occurs in nature in a number of species of the genus *Hebe*. In a population of *H. Traversii* at Cass, New Zealand, 3.5 % of a total of 2200 plants were found to be pollen sterile (Frankel & Hair, 1937). A survey in other species was confined to one or more specimens which had been obtained for cytological studies. In this material, pollen sterility was found in seven out of fifteen plants of *H. subalpina*, obtained from seven localities; in two plants each of *H. Traversii* and *H. Townsoni*; in one each of *H. parviflora* and *H. salicifolia* var. *communis*; and in a natural hybrid *H. salicifolia* \times *H. leiophylla*. These observations, though by no means exhaustive, may be taken as an indication of a high incidence of pollen sterility, at least in some species of this genus.

Hebe is unsuited for genetic study, since plants reach the flowering stage at the earliest in their third year, and usually later. Hence the

genetic nature of male sterility has not been investigated. That this defect is due to genetic and not to environmental causes can however be concluded from the precision of the mechanisms of degeneration and from the regularity of the phenomena from year to year and in a new environment after transplantation. That it occurs mainly in small, isolated populations suggests that it is due to the action of recessive genes.

All but one of the plants used in this study set seed when open-pollinated or crossed with fertile plants of their own species, but failed to set seed when fertilization was prevented. The one exception, *H. Townsoni*, flowers so early that no pollen of related forms was available for fertilization.

MATERIAL AND METHODS

The following plants were used in this study:

No.	Species	Chromosome number	Origin
—	<i>H. parviflora</i> (Vahl.) Ckn. & Allan	80*	Christchurch Botanical Gardens
HV 46)	<i>H. subalpina</i> (Ckn.) Ckn. & Allan	80*	Arthur's Pass
HV 211]			
HV 338			
HV 370	<i>H. Traversii</i> (Hook. f.) Ckn. & Allan	—	Waikouiti River
	<i>H. salicifolia</i> var. <i>communis</i> (Ckn.) Ckn. & Allan	40	Pelorus Sound
HV 120	<i>H. salicifolia</i> × <i>H. leiophylla</i>	40	Fox Creek, Okuku
HV 225	<i>H. Traversii</i> (Hook. f.) Ckn. & Allan	120	Ghost Creek, Castle Hill
HV 157	<i>H. Townsoni</i> (Cheeseman) Ckn. & Allan	40	Hills near Westport

* Chromosome number of fertile plants of the same species.

Meiotic divisions were studied on paraffin material fixed in Allen's Bouin. Both this fixative and aceto-carmin were used for the study of pollen grain development. Drawings of pollen grains are from aceto-carmin material.

PROPHASE DEGENERATION

Hebe parviflora. Up to pachytene meiosis is normal. Owing to the large number of chromosomes, details of pachytene pairing cannot be reliably observed, but no unpaired or partly paired threads have been seen. Degeneration occurs at this stage; diplotene loops are not formed. The pachytene threads coagulate individually, approximately to the size of diakinesis bivalents (Fig. 1), and collapse into an amorphous mass which is rapidly dissolved. Subsequently the empty cell walls collapse but remain in this state in the anther cavity. The tapetum cells degenerate simultaneously with the pollen mother cells.

The principal features of this process of degeneration are its rapidity and its regularity. Development in normal loculi of *Hebe* proceeds in an orderly manner, with the central region slightly in advance of the distal ones. This rhythm is reflected in the incidence of degeneration; normal pachytene and degenerating cells can be found within one loculus. But the process is so rapid that the transitional stages are only rarely seen. Whilst in *Lathyrus odoratus* the incidence of degeneration varies at random from cell to cell (Fabergé, 1937), in *Hebe parviflora* it is strictly synchronized in large blocks of tissue. It is progressive within loculi, and, as may be concluded from the rarely seen stage illustrated in Fig. 1, even within cells.

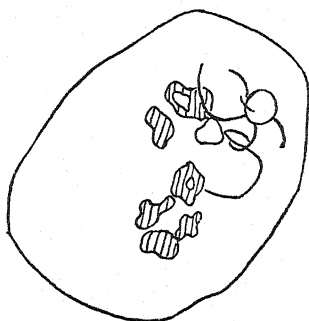


Fig. 1. Earliest stage of pachytene degeneration in *Hebe parviflora*. $\times 2500$.

H. subalpina (HV 46 and 211). Degeneration occurs in pachytene along the lines described for *H. parviflora*. The only distinguishing feature is the complete disappearance of cell walls of pollen mother cells and tapetum cells. This is accomplished very rapidly, at the same time as zygotene in the megaspore mother cell, *Hebe* being slightly proterandrous.

H. Traversii (HV 338). Degeneration occurs during meiotic prophase. No details have been observed.

TETRAD DEGENERATION

H. salicifolia var. *communis* (HV 370). All meiotic stages up to telophase II are normal. There is a high degree of terminalization of chiasmata, as is usual for all species of *Hebe*. Tetrads are formed but collapse rapidly; no pollen grains have been seen.

POLLEN DEGENERATION

H. leiophylla \times *H. salicifolia* (HV 120). In a population of *H. salicifolia* var. *communis*, a plant was found in various characters intermediate between this species and *H. leiophylla*. Some plants of the latter were

situated within a short distance. Plants similar to HV 120 and found under comparable circumstances had been described by Allan (1925) as natural hybrids. Both alleged parents and HV 120 have identical chromosome numbers (Frankel & Hair, 1937; and Frankel, unpublished). This plant is therefore interpreted as a natural hybrid, of unknown status, between *H. salicifolia* and *H. leiophylla*.

Metaphase I bivalents show the features characteristic of normal forms of *Hebe*, viz. a prevalence of rod bivalents with a single terminal or sub-terminal chiasma, and a small number of ring bivalents. There is the high degree of terminalization typical of the genus. The tetrad cells round off, and about a day later, not having undergone a change either in diameter or in wall structure, the pollen grains shrivel, collapse and gradually

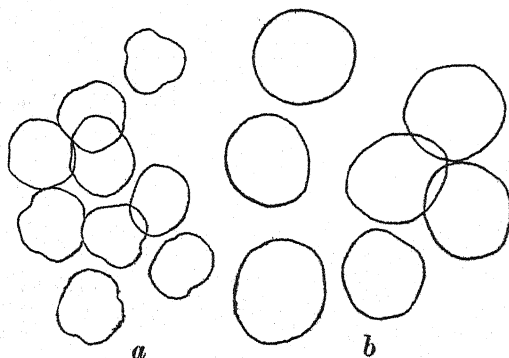


Fig. 2. Pollen degeneration in HV 120. *a*, newly formed pollen grains; *b*, degenerating pollen. $\times 465$.

disintegrate. This process is as rapid and regular as that of pachytene degeneration described above. Concurrently the tapetum cells degenerate. There is no indication of an impending pollen-grain division at the onset of degeneration.

The pollen development of HV 114, a normal plant of *H. leiophylla*, was studied for comparison. In *Hebe* the development of the inflorescence takes place in a well-defined order starting from the lowest whorl. After pollen grains are formed, their average diameter is 18.7μ , which is identical with that of newly formed grains of the sterile plant HV 120. The next older whorl—no further removed than one day—has pollen grains of a diameter of 26.3μ . Simultaneously the cell wall thickens. The nucleus is still undivided. There is little change until flowering, when the diameter is 27.8μ . In HV 120 degeneration occurs at a stage corresponding to the

enlargement in HV 114, i.e. one whorl above the unenlarged pollen grains (Fig. 3).

H. Traversii (HV 225). Both meiotic divisions are normal throughout. As is usual in the hexaploid form of this species (Frankel & Hair, 1937), some quadrivalents are formed. One day after the formation of pollen grains, they increase in size and the cell wall is slightly thickened. On the

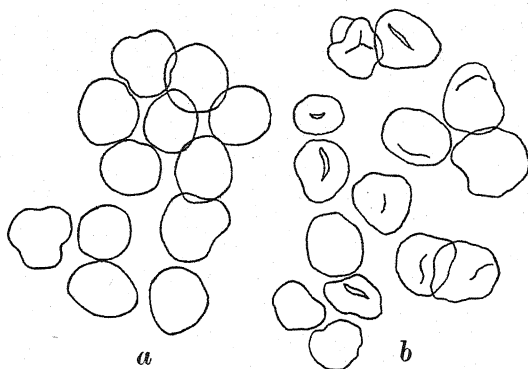


Fig. 3. Normal pollen development in HV 114. *a* and *b*, corresponding to Fig. 2 *a* and *b*. $\times 465$.

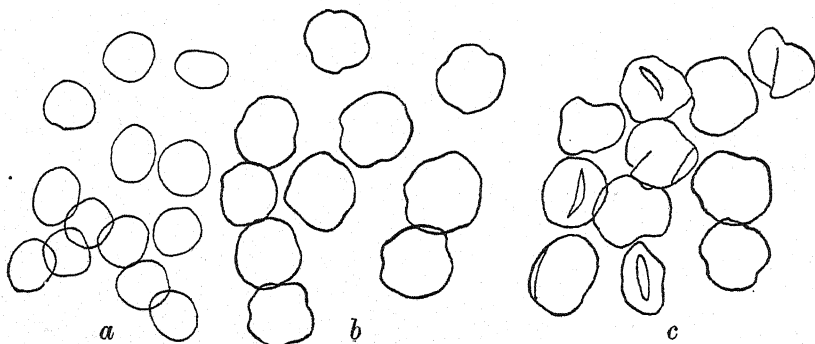


Fig. 4. Pollen degeneration in *H. Traversii* HV 225. *a*, newly formed pollen; *b*, thickening of wall; *c*, collapse. $\times 465$.

following day, if not sooner, shrivelling sets in, leading rapidly to a collapse of pollen grains (Fig. 4) and tapetum, both of which almost disappear. The anthers consequently are shrivelled.

A comparison with the normal plant HV 89 shows that degeneration occurs prior to the main thickening of the cell wall and at least one week before the pollen grain division. In this plant pollen grains grow more gradually than is the case in *H. leiophylla*. Their diameter at first is

18.8 μ ; the thickening of the wall sets in at 24 μ —when degeneration occurs in HV 225—and is completed at 32 μ . Mature grains are 35 μ in diameter.

H. Townsoni (HV 157). Metaphase pairing is normal, with the exception of one bivalent which frequently fails to pair (Figs. 5, 6). Among 215 cells in anaphase I, fourteen showed two univalents and one cell one univalent, i.e. 7 % of the cells contained visible univalents, far in excess of

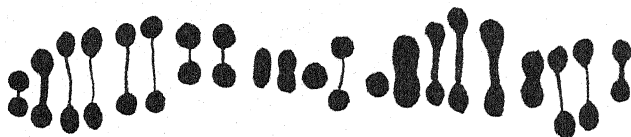


Fig. 5. *H. Townsoni* HV 157. Metaphase I. 19^{II} and 2^I. $\times 4500$.

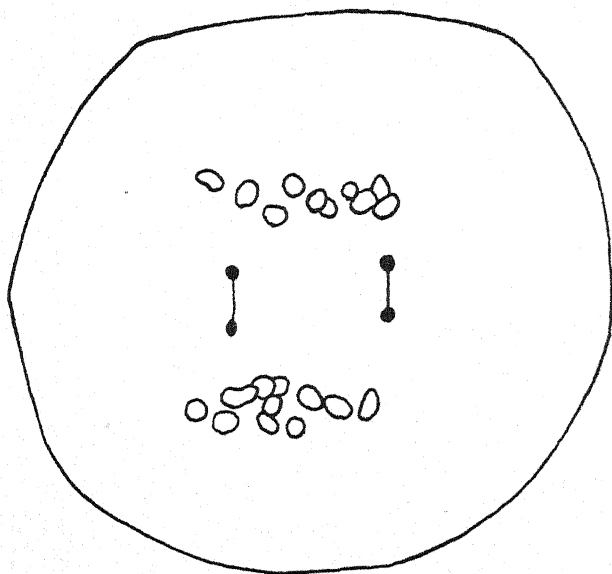


Fig. 6. *H. Townsoni* HV 157. Anaphase I. Two dividing univalents. $\times 4500$.

the sporadically occurring failures of pairing in normal forms of *Hebe*. Normal tetrads and pollen grains are formed. The latter enlarge slightly but presently show signs of collapse (Fig. 7). Thus they remain, without any further disintegration, up to the opening of the flowers. The anthers fail to dehisce.

Normal anthers of *Hebe* exhibit a well-defined inter- and intralocular variation in meiotic development. Within loculi, this variation is strictly limited in scope; the distal ends lag slightly behind the central regions,

the difference in development never exceeding such closely related stages as metaphase I—interphase, or metaphase II—teophase II. Early prophase stages never occur in the same loculus with metaphase I. Variation in transverse sections of loculi is confined to such narrow limits as late metaphase—early anaphase.

The male-sterile plant of *H. Townsoni* is the only plant in *Hebe* in which a disturbance of regular timing in meiosis has been observed. The general trend is maintained, the central region of a loculus being ahead

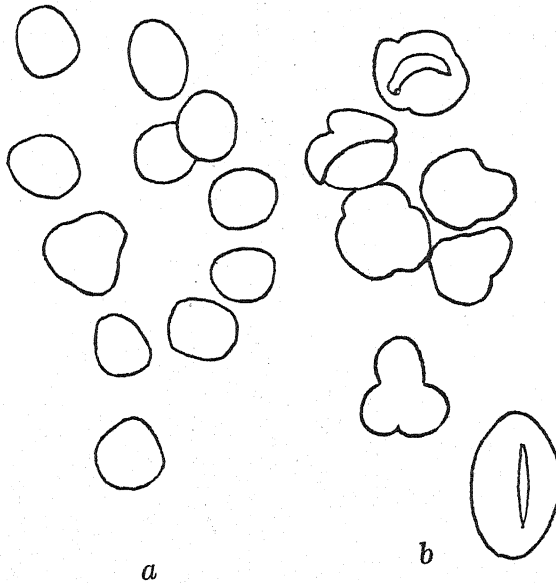


Fig. 7. *H. Townsoni* HV 157. Pollen degeneration. *a*, newly formed pollen; *b*, collapsing pollen grains. $\times 700$.

of the ends in its meiotic development; but (1) the scope of timing variation greatly exceeds the norm, and (2) there is a chimerical arrangement of division stages throughout the loculus. Fig. 8 illustrates a longitudinal section through a typical loculus. All stages from pachytene to interphase are represented, in blocks ranging in size from a few cells to one-quarter of the loculus. The blocks are sharply defined, without transitional stages; interphase may border on pachytene (Fig. 9). By the time the tetrads are formed, variation has largely disappeared. A few isolated metaphase II cells, however, have been seen among tetrads (Fig. 10).

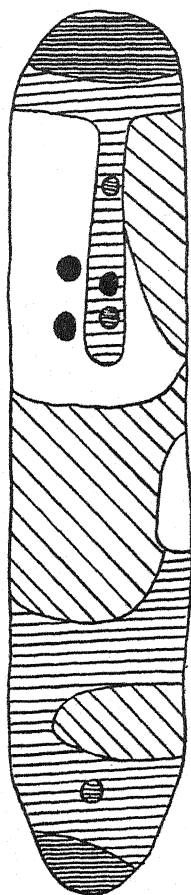


Fig. 8. Longitudinal section through a loculus of *H. Townsoni* HV 157. ■ pachytene-diplotene; ▨ diakinesis; ▤ metaphase I; ▥ anaphase I; □ interphase.

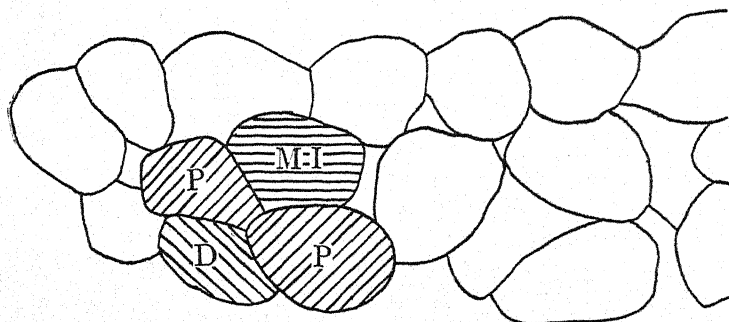


Fig. 9. Cross-section through a loculus of *H. Townsoni* HV 157, containing pachytene (P), diplotene (D), metaphase I (MI) and interphase (white). $\times 1130$.

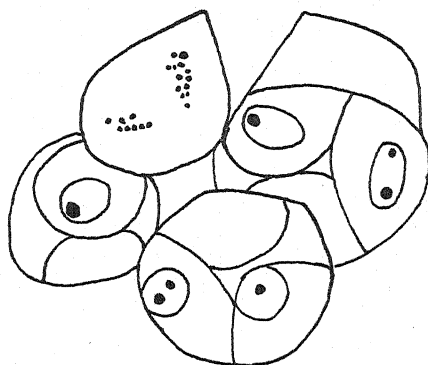


Fig. 10. *H. Townsoni* HV 157. Metaphase II among tetrads. $\times 1700$.

DISCUSSION

(1) "Undefined" male sterility

Genotypic control inhibiting the production of functional male gametes in hermaphroditic plants may be clearly "defined" in its influence on chiasma or spindle formation (cf. Darlington, 1937); or "undefined", it may cause destruction at varying stages either of meiosis or of pollen grain development. Excluding *H. Townsoni* from the first part of this discussion, the observations recorded above range male sterility in *Hebe* among the second group. Degeneration occurs either after the completion of normal pachytene pairing, or at various stages of pollen development following a normal meiosis. In the latter group there are no indications of disturbance in the formation or in the movement of chiasmata, either in number or in time (cf. Upcott, 1937; Fabergé, 1937). Whereas variation from cell to cell is the usual consequence of irregular chromosome behaviour, the processes of development and of degeneration in *Hebe* exhibit a striking regularity. The unit of variation is not the individual bivalent or cell, but the loculus, or, within narrow limits, blocks of tissue within it.

There are two phases at which degeneration occurs in *Hebe*, pachytene and various stages of pollen development preceding the first pollen grain division. In each male-sterile form the critical phase is clearly defined and open to a minimum of variation.

Types of male sterility similar to those reported above have been observed in a number of plants. Male-sterility genes not associated with

irregular chromosome or spindle behaviour have been studied cytologically in maize (Beadle, 1932), in *Sorghum*¹ (Hadjinov, 1937), in *Lolium* (Thomas, 1937) and in *Canna* (Offerijns, 1938). An unnamed gene in maize causes degeneration in synizesis or pachytene, one in *Canna* between pachytene and diakinesis. One gene in maize (m_{s4}) and one in *Sorghum* affect the pollen grains before thickening of the wall, two in maize (m_{s10} and m_{s13}) at the beginning of thickening, four (m_{s1} , m_{s5} , m_{s7} , m_{s14}) after thickening of the wall but before the first division, and one (m_{s3}) after the nucleus in some cells has undergone this division. In a case of cytoplasmically inherited male sterility in maize (Rhoades, 1933), degeneration of pollen grains occurs as a rule before the first pollen grain division, but some cells complete their development to become functional pollen grains. In *Lolium perenne*, where data recorded by Jenkin (1931) suggest a similar maternal inheritance, Thomas (1937) found also degeneration before the first pollen-grain division. In all these plants the two main phases of degeneration, pachytene and pollen development, correspond to those observed in *Hebe*. In contrast to the regularity of behaviour in this genus, some variation in the stage of degeneration occurs in the majority of the male-sterile forms in maize, being specially marked in two genes (m_{s8} and m_{s9}) which cause degeneration between pachytene and pollen-grain formation, and in m_{s6} , a partially sterile form, with degeneration occurring between metaphase I and pollen maturity. In *Canna* all pollen mother cells of some loculi are affected, whilst others produce only normal pollen. This type of interlocular variation may be essentially analogous to the more common type of intercellular variation in variable-sterile forms. In both cases variation may be the effect of interactions between the gene and the environment, with the critical period occurring at different developmental stages. The regularity of timing in the processes of degeneration in male-sterile *Hebe* may then be understood as due to an uncommonly regular development, or to a high degree of buffering of the male-sterile genes.

No attempt has been made in this study or elsewhere to investigate the physiology of male sterility. Beadle (1932) assumes that "degeneration takes place...because of some sort of nutritive disturbance". In maize, as in *Hebe*, the tapetal cells degenerate simultaneously with the pollen mother cells or young pollen grains. For a study of genic influence on the development of the male gametes the pollen-sterile forms of *Hebe*,

¹ It is doubtful whether this case belongs to this group, since structural hybridity as a consequence of intervarietal hybridization cannot be excluded as a possible explanation of reduced fertility, equally in male and in female gametes.

with their regularity of behaviour in each form, but interspecific variation in the time of incidence of degeneration, may be useful material.

(2) *Male sterility in H. Townsoni: timing unbalance*

H. Townsoni differs from all other male-sterile forms of *Hebe* in three respects:

(a) There is a timing unbalance in meiosis between blocks of cells within loculi. Timing unbalance expresses itself as delayed meiotic development correlated with increased spiralization and terminalization and leads eventually to male sterility in *Lathyrus odoratus* (Upcott, 1937). No such delayed anther development occurs in *Hebe Townsoni*. Terminalization is normal, nor could it go far beyond the degree characteristic of normal plants. Whether chiasma formation is reduced could not be ascertained, in view of the large number and small size of diplotene bivalents. Metaphase I chiasma frequencies are normal. The most striking difference from *Lathyrus*, however, is found in the strict localization of timing differences, which causes a patchwork of timing phases within loculi. If a timing disturbance at an archesporial division were the cause of localized delay, partial rather than complete sterility might be expected to follow. The assumption seems justified that the timing differences in meiosis are the effect rather than the cause of a more general physiological disturbance whose nature at present cannot be defined.

(b) There is a frequent failure of pairing of one bivalent in metaphase I. It cannot be ascertained whether this is due to a general reduction in chiasma formation affecting all chromosomes or to causes specifically affecting one bivalent. The assumption seems justified that timing and pairing unbalance are associated with male sterility, though causal relationships are unknown.

(c) The shrunken pollen grains remain intact without further collapse and disintegration. This type of behaviour differs not only from that of other male-sterile forms in *Hebe*, but also from that in most other male-sterile plants.

(3) *The occurrence and significance of male sterility in nature*

Male sterility has mostly been seen in cultivated plants where large numbers of individuals have been closely studied, and in particular where self-fertilization is artificially induced. In such circumstances a great variety of mechanisms causing pollen sterility have been found. It ap-

pears widespread, since apart from the examples quoted—*Zea*, *Sorghum*, *Canna*, *Lolium*, *Lathyrus*—it has been found in seedlings of peaches, plums, pears (Crane & Lawrence, 1938) and in strawberries.

In nature it has hardly been acknowledged. Yet there is reason to believe that it cannot be uncommon. It might be determined by cytoplasmic reaction, as in one case in maize and possibly in *Lolium*, and in species crosses in *Linum* and *Epilobium* (cf. Darlington, 1939); or it might be inherited as a simple recessive which would appear after inbreeding. In the latter case it should occur primarily in small populations where inbreeding is favoured. This doubtless is the case in the genus *Hebe*. Swarms of various sizes, from a few plants to several thousand, occur in burned forest areas, shingle slips, tussock lands, river beds, lake shores and roadsides. Single plants, separated by considerable distances from others of the same or related species, are not uncommon. Furthermore, nearly all the plants tested for self-fertility produced seed on selfing (Frankel & Hair, 1937). Such conditions favour the production of male-sterile plants if, as is likely, male sterility is inherited as a recessive. It is obvious that under such circumstances male sterility has a distinct physiological advantage in preventing a prevalence of self-fertilization, and that a balance between pollen fertility and sterility should exist which would be best adapted to the group requirements, just as the sex ratio is in dioecious species. The maintenance of male sterility, once it is established in any species, will depend on the balance between such factors as self- and cross-sterility, relative vigour in consequence of either system of fertilization, population density and flowering mechanism. Where, as in the first group of male-sterile *Hebe*, degeneration is highly regular, one may assume that the male sterility is old established and well adapted to the requirements of the species, and that the genes responsible are highly buffered. Timing disturbances such as occur in *H. Townsoni*, on the other hand, indicate a more recent origin and less stable foundation.

It seems likely that in other genera where similar conditions prevail, male sterility occurs but has not been noticed, the detailed observation required lying as a rule outside the scope of systematic or field observation. It is not unlikely, moreover, that many plants which have been recorded as gynodioecious, as for example many Labiatae (cf. Yampolsky, 1922) are in effect species in which a certain proportion of individuals show male-sterile inheritance. One may be justified in expanding Clausen's (1930) suggestion that "male-sterile types really mark a step on the way to the differentiating out of gynodioecious races" by suggesting that male sterility, rather than being a step towards gynodioecism,

is the mechanism by which "female" plants in "gynodioecious" forms are reproduced.

ACKNOWLEDGEMENT

I am indebted to Dr C. D. Darlington, who read the manuscript and made valuable suggestions.

SUMMARY

1. Male sterility with female fertility has been observed in nature in eight forms of the genus *Hebe*, comprising five species and one species hybrid.

2. Degeneration occurs rapidly and with regularity, either in pachytene or in the course of pollen development, at a stage which is characteristic for each form.

3. In *H. Townsoni* male sterility is associated with a timing disturbance which causes a chimerical arrangement of division stages within loculi, and also with a frequent failure of pairing of one bivalent. A causal relationship cannot be ascertained. The three phenomena may indicate a major physiological disturbance.

4. Male sterility has mainly been ascertained under experimental conditions, especially after induced self-fertilization. Under conditions of relative isolation, which favours its occurrence, male sterility serves as a mechanism for reducing self-fertilization.

5. In gynodioecious species, the production of "female" plants may often, originally, be determined by single genes for pollen sterility.

REFERENCES

- ALLAN, H. H. (1925). "Illustrations of hybrids in the New Zealand flora." *Genetics*, **7**, 287-92.
- BEADLE, G. W. (1932). "Genes in maize for pollen sterility." *Genetics*, **17**, 413-31.
- CLAUSEN, J. (1930). "Male sterility in *Viola orphanidis*." *Hereditas, Lund*, **14**, 53-72.
- CRANE, M. B. & LAWRENCE, W. J. C. (1938). *The Genetics of Garden Plants*, 2nd ed. London.
- DARLINGTON, C. D. (1937). *Recent Advances in Cytology*, 2nd ed. London.
- (1939). *The Evolution of Genetic Systems*. Cambridge.
- FABERGÉ, A. C. (1937). "The cytology of the male sterile *Lathyrus odoratus*." *Genetica*, **19**, 423-30.
- FRANKEL, O. H. & HAIR, J. B. (1937). "Studies on the cytology, genetics and taxonomy of New Zealand *Hebe* and *Veronica*. I." *N.Z. J. Sci. Tech.* **18**, 669-87.
- HADJINOV, M. I. (1937). "Sterility in varietal hybrids of *Sorghum*." *Bull. Appl. Bot. Gen. Pl. Breed.* series II, no. 7, pp. 442-6.

- JENKIN, T. J. (1931). "Self-fertility in perennial rye grass." *Bull. Welsh Pl. Breed. Sta.* **12**, 100-19.
- OFFERIJNS, F. J. M. (1938). "Meiosis in the pollen mother cells of *Canna glauca*, 'Pure Yellow'." *Genetics*, **20**, 59-65.
- RHOADES, M. M. (1933). "The cytoplasmic inheritance of male sterility in *Zea Mays*." *J. Genet.* **27**, 71-93.
- THOMAS, P. T. (1937). "The cytology of certain interspecific and intergeneric hybrids in the *Lolium-Festuca* group." Ph.D. Thesis, Aberystwyth.
- UPCOTT, M. (1937). "Timing unbalance at meiosis in the pollen-sterile *Lathyrus odoratus*." *Cytologia, Tokyo*, Fujii Jubilee Volume, pp. 299-310.
- YAMPOLSKY, C. & H. (1922). "Distribution of sex forms in the phanerogamic flora." *Biblioth. genet.* **3**, 1-62.

NUCLEIC ACID STARVATION OF CHROMOSOMES IN *TRILLIUM*

By C. D. DARLINGTON AND L. LA COUR

John Innes Horticultural Institution, Merton

(With Plates VII-IX and Fourteen Text-figures)

CONTENTS

	PAGE
1. Differential activity in <i>Paris</i>	185
2. The threshold for differentiability in <i>Trillium</i>	186
3. Metaphase: variation and hybridity	188
4. Anaphase: the reproductive error	196
5. Resting stage: reversibility of the reaction	199
6. Supply and demand of nucleic acid	201
7. Allocyclus and gene reproduction	206
8. Summary	209
References	210
Explanation of Plates VII-IX	213

1. DIFFERENTIAL ACTIVITY IN *PARIS*

PARIS POLYPHYLLA, a species of the order Liliaceae, has five pairs of the largest chromosomes known. They range from 22 to 36 μ in length at mitosis. Under ordinary conditions their chromatids have a uniform staining capacity and they also have a uniform diameter, apart from the usual constrictions at the centromeres and at points of nucleolus formation.

Under special conditions this uniformity breaks down. Certain segments in each chromosome are reduced to half the standard diameter of the chromatid and appear under-stained from late prophase until anaphase. They remain their usual length. This difference is clear not only with gentian violet staining but also with the specific Feulgen reaction. We therefore conclude that these segments are under-charged with nucleic acid (Darlington & La Cour, 1938).

The special segments are (so far as we have seen) constant in size and position throughout the root tips of the individual. Their reduced activity, or abnormal cycle, with respect to nucleic acid is specific to the genes and groups of genes comprising them. Furthermore, since the individual studied was hybrid for a deficiency (or duplication) in one of these segments it seemed possible that they were less active in what they

186 *Nucleic Acid Starvation of Chromosomes in Trillium*

gave up to the nucleus as well as in what they took out of it. This possibility was supported by the regular position of the inactive segments. They were all terminal and lay at the far ends of the chromosomes from the centromeres: in a region, that is, from which crossing-over is largely excluded. As in *Paris quadrifolia*, pairing is procentric and crossing-over localised (Darlington, 1937, 1940).

Genetic inertness is characteristic of segments which are over-charged with nucleic acid in the resting stage. Such segments are described as heterochromatic (Heitz, 1935) and, as we shall show later, our under-charged segments may be assigned to the same class.

The special segments are remarkable in two respects. They are the only example of under-charged segments in plants. Such segments occur in the X-chromosomes of certain animals. For example, they are seen in many Orthoptera at the mitoses preceding meiosis. They are also found at the first metaphase of meiosis itself in mammals. This behaviour is somewhat variable. It is not usually unconditional. In one mammal, *Cricetus*, however, it reaches such an extreme degree as to appear invariable (Koller, 1938).

This brings us to the second point. The reduced activity of the special segments in *Paris* is conditional. In most preparations no differential behaviour is seen. Our first task now is to find out how this conditioning works. We shall then be able to apply it as a genetic and taxonomic indicator, an indicator of variation and hybridity within and between species. And finally we shall be able to enquire into its physiological meaning by comparison with abnormalities of the nucleic acid cycle elsewhere.

2. THE THRESHOLD FOR DIFFERENTIALITY IN *TRILLIUM*

We originally suggested that temperature was the condition of revealing differential activity. We now find that the special segments regularly appear in the root tips of *Paris polyphylla* after 2 days at 0° C. Whether it is shorter treatment or higher temperature that allows of the intermediate expressions we have illustrated we do not yet know.

This minimum period of 2 days probably depends on the length of the mitotic cycle of the plant at the temperature in question. It has failed to produce an effect in several other species. We have however tried 5 days of treatment at zero on a number of other plants. This has had the diverse effects shown in Table I.

We find, as we should expect, that, at a temperature at which mitosis is stopped in some species, it continues in others but with varying

degrees of abnormality. The characteristic symptom of upset is shown by *Allium Cepa* where the chromosomes are superspiralized in most of the metaphases seen. They are contracted to the same degree as in meiosis and presumably develop major spirals. Super-contraction from cold treatment has also been illustrated in *Crepis* by Delaunay (cf. Darlington, 1937). This condition goes with a breakdown of the spindle. No doubt, when no spindle develops, the chromosomes continue to spiralize after the disappearance of the nuclear membrane. The chromatid attraction lapses but without a spindle the explosion of the centromeres and the anaphase movement are impossible.

TABLE I

Effects on mitosis of 5 days at freezing-point

	1. Mitosis ceases	2. Spindle upset	3. Mitosis normal	4. Differential activity
Root tips	<i>Tradescantia bracteata</i> <i>Paeonia Veitchii</i>	<i>Allium Cepa</i> <i>Uvularia</i> <i>Convallaria</i> <i>Galanthus</i> <i>Narcissus</i> <i>Paris japonica</i>	<i>Fritillaria Meleagris</i> <i>Lilium Martagon</i> <i>Hyacinthus orientalis</i> <i>Paeonia Delavayi</i> <i>P. anomala</i>	All species of <i>Paris</i> and <i>Trillium</i>
Other tissues	—	—	<i>Tradescantia bracteata</i> (pollen grain)	<i>Trillium erectum</i> <i>T. kamtschaticum</i> (ovary walls)

Notes. (1) Mitosis continues at -5°C . in *Trillium*, and at -10°C . in *Fritillaria*, but continuation of this temperature kills the *Fritillaria* bulb. It is significant that seeds of *Paris polyphylla* require a low temperature for germination, freezing-point being, it seems, the optimum temperature.

(2) The fixation and staining methods (2BD and Feulgen) were those described in the appendix to our previous paper, except that acid fumes were not used before fixation. The time of hydrolysis used was always 16 minutes, the extra 8 minutes being necessary to soften the tissues (cf. Hillary, 1939).

The correlation of supercoiling and spindle breakdown is thus a natural one. It arises regularly after colchicine treatment in *Allium* and elsewhere (Levan, 1938). The marginal temperature and the drug equally produce cell anaesthesia without chromosome anaesthesia, whence their use in doubling the chromosome number in the cell.

In *Paris* and *Trillium* there is no cell anaesthesia even at -5°C . The possibility cannot be excluded that in other genera the temperature threshold for differentiability may be so close to the threshold for mitosis itself that another technique would be required for showing it. Nor can we be sure that differential segments are not present in many species

188 *Nucleic Acid Starvation of Chromosomes in Trillium*

where they are too small to be seen. It should be noted too that the failure of previous observers to obtain a differentiation of the metaphase chromosomes at low temperatures is inconclusive (e.g. Ellenhorn, 1934) since they have not given their plants long enough to pass through a whole mitotic cycle at the low temperature.

Our classification of the effects of cold thus shows a development of differential activity only in the tribe Paridae. Elsewhere the chromosomes either have smaller differential segments, or no differential potentiality, or a lower threshold for showing it¹.

3. METAPHASE: VARIATION AND HYBRIDITY

Several species of *Trillium* were examined. The numbers of plants from each source were as follows:

North American species:

<i>T. grandiflorum</i>	Hocker Edge Gardens	7 plants
	R.H.S., Wisley	1 plant (no. 6)
	C. G. van Tubergen	1 plant (no. 1)
<i>T. sessile</i>	C. G. van Tubergen	3 plants
<i>T. recurvatum</i>	Dr E. J. Kraus, Chicago	2 plants
	(collected)	
<i>T. stylosum</i>	C. G. van Tubergen	3 plants
<i>T. erectum</i>	Hocker Edge Gardens	1 plant

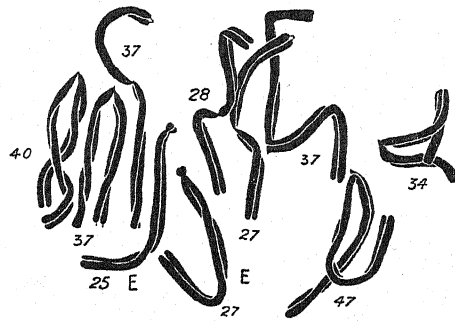
Asiatic species:

<i>T. kamtschaticum</i>	Hocker Edge Gardens	1 plant
-------------------------	---------------------	---------

These species are not recognizably different in chromosome complement under ordinary conditions. They all have five pairs of chromosomes which are of two distinct types: four have their centromeres submedian and one subterminal. The submedian types differ in length, but, as we shall see later, any classification on this basis, such as Warmke found possible in the west coast species, would be misleading (Text-fig. 1).

The low temperature preparations, on the other hand, enable us to recognize each type of chromosome (*A* to *E*) in all the species. Comparison of normal and treated material in *T. stylosum*, *T. grandiflorum* and *T. sessile* (Text-figs. 1, 2, 4-6) shows that this depends, as in *Paris*, on a reduction in staining capacity and diameter in the differential segment of the chromatid, without a change in its length. One reservation must

¹ We have later found differential segments at metaphase in chilled triploid *Fritillaria pudica*, both in root-tip and pollen grain mitoses. They do not appear in *Trillium* pollen grains after a week's treatment owing, we believe, to the longer resting stage before the observed mitosis.



Text-fig. 1. *T. grandiflorum* 7, early mitotic metaphase at normal temperature, showing lengths of separate chromosomes in microns. Two chromosomes have trabants, none have differential staining (cf. Fig. 6). *E* is alone in having a subterminal centromere in all species. All illustrations are from root-tip Feulgen smears, except where otherwise stated. $\times 1000$.

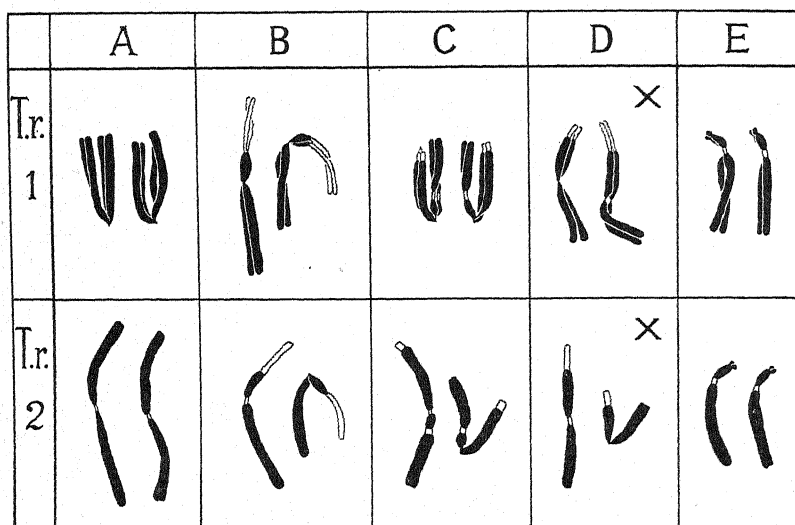
	A	B	C	D	E
1 _a					
1					
2					

Text-fig. 2. *T. sessile*, plants 1 and 2, metaphase chromosomes, 1_a normal, 1 and 2 low temperature, classified in types *A* to *E*, with under-charged or heterochromatic segments in outline. *X* in this and the following figures indicates a hybrid pair. $\times 1000$.

190 Nucleic Acid Starvation of Chromosomes in Trillium

be made. The length of the chromosomes, depending on the degree of spiralization, is more variable from cell to cell at this marginal temperature than under normal conditions (*e.g.* Text-fig. 4, chromosome pair 3c).

Taking now the first plant of *T. sessile*, we see that *A* and *E* have no differential segments, while three pairs of chromosomes, *B*, *C* and *D*, show these segments on one arm only. These segments, as in *Paris polyphylla*, are all terminal. Again, as in *Paris*, they show hybridity. In both *C* and *D* they are of unequal length.

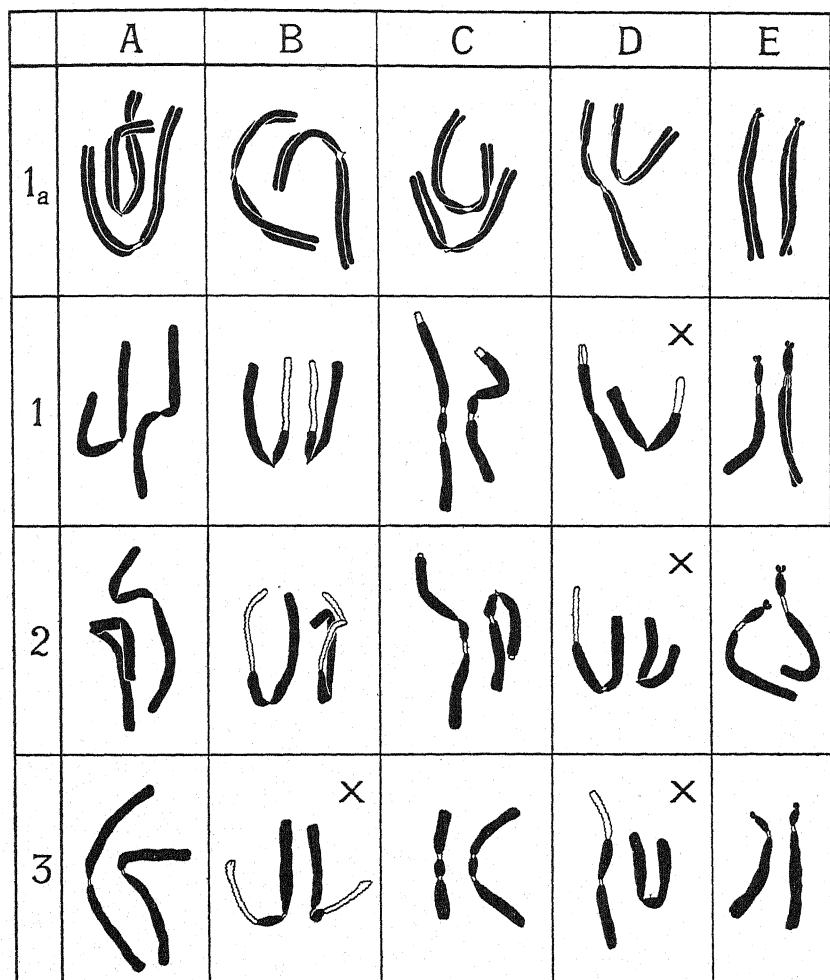


Text-fig. 3. *T. recurvatum*, chilled chromosomes of two plants, each hybrid in *D* and indistinguishable, therefore a clone. $\times 1000$.

When we turn to the second plant we find a similar condition of hybridity in *D*. But we also find two new conditions. The first is a differential segment in *C* and *E* which is intercalary and in a corresponding position in each pair. This segment is so small as to seem little more than a secondary constriction. Such a segment may well explain the long or excessively numerous secondary constrictions in other Liliaceae, *e.g.* *Lachenalia* (Moffett, 1936) and *Aloë striatulus* (Resende, 1937). Indeed it might be thought that all secondary constrictions other than those due to nucleolar organizers are intercalary segments of the kind we are describing.

The second new condition in this plant is the appearance of a small normal segment intercalated within the differential segment in both *B*'s and represented by a brightly staining point in each under-stained chromatid (Text-fig. 2).

T. sessile thus shows us the two expected symptoms of variation, differences between homologous chromosomes both of different individuals and of the same individual.

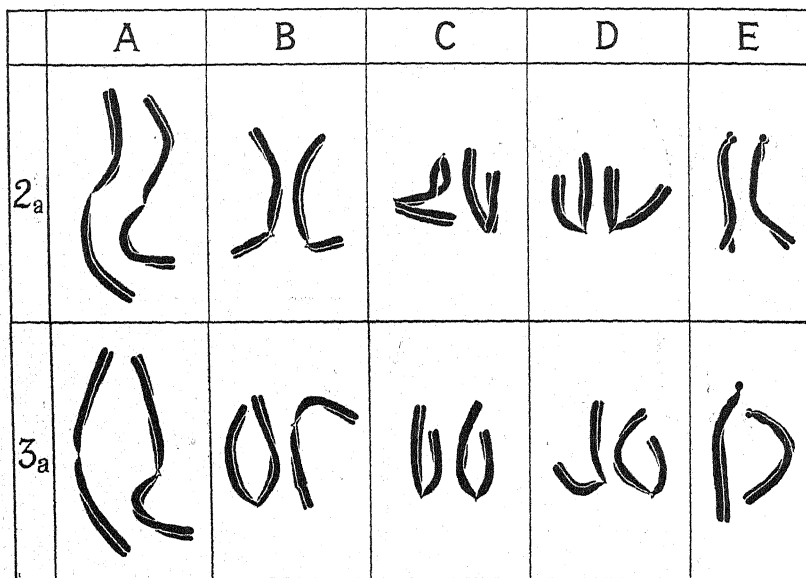


Text-fig. 4. *T. stylosum*, chilled chromosomes of three plants. 1a shows the normal unchilled chromosomes of the first plant for comparison. $\times 1000$.

The two plants of *T. recurvatum* are apparently identical and, since they are also hybrid, we need not doubt that they are part of the same clone collected in nature. They closely resemble the second plant of *T. sessile* except for the absence of the intercalary granule in B (Text-fig. 3).

192 *Nucleic Acid Starvation of Chromosomes in Trillium*

The three plants of *T. stylosum* resemble *T. recurvatum* in general, but have longer differential segments both intercalary and terminal. Again hybridity is found in both *B* and *D* chromosomes. The second and

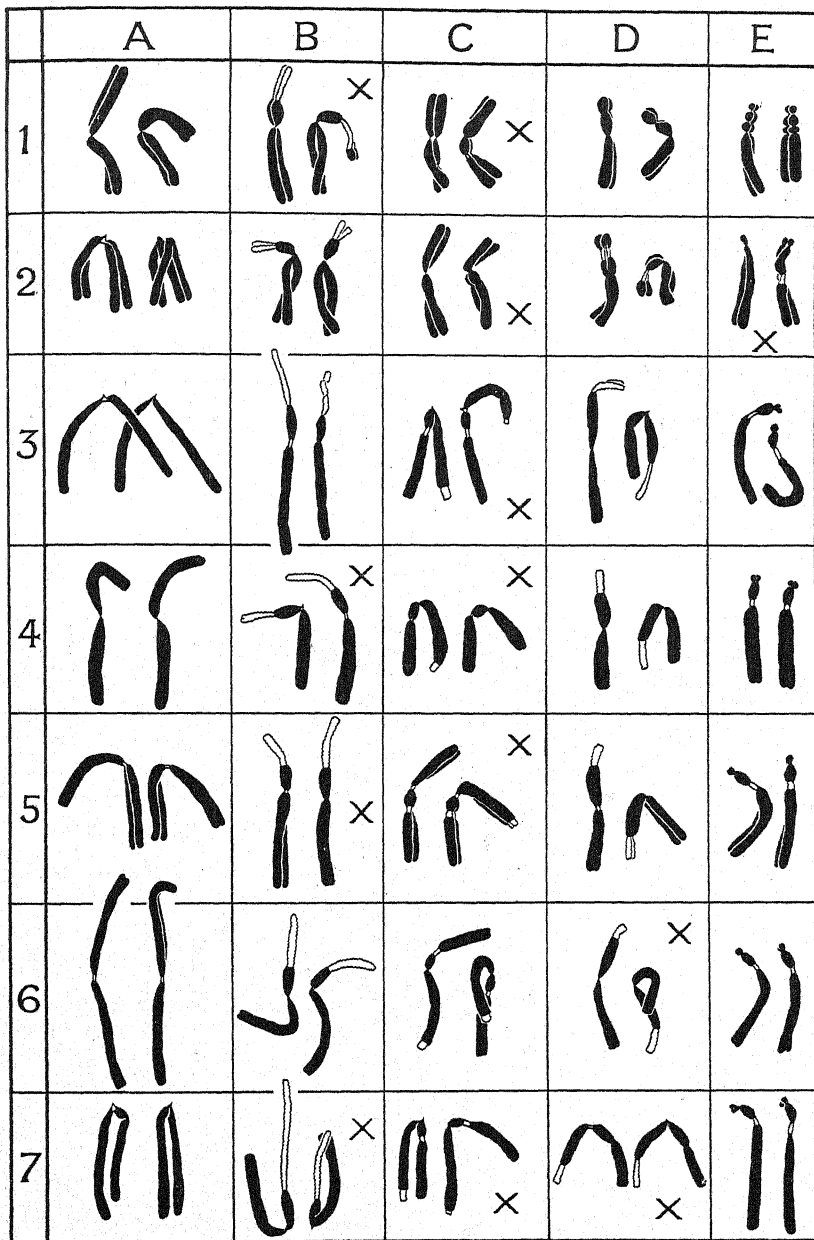


Text-fig. 5. *T. grandiflorum*, normal chromosomes of plants 2 and 3. $\times 1000$.

third plants differ only in the presence of a small terminal segment in *C* (Text-fig. 4).

In *T. grandiflorum* we have been able to make the most extensive comparison. There is no constant difference between this and the other species, but several novelties appear. One *B* of plant 1 is remarkable for the longest intercalary differential. Hence the enormous deficiency possible in the partner of this chromosome and detectable even in unchilled material (Text-fig. 5). In *C*, *D* and *E* chromosomes we find differentials close to the centromere. Some are long and others are short and repeated. In regard to these segments the first two plants are sharply distinct from the rest.

Our single plant of *T. erectum* has the most elaborate combination of differential segments that we have found. We have therefore used the complement for the comparison of mitosis in different tissues (Text-fig. 7). This plant is the first to show differential segments in *A*. Every chromosome except *D* is hybrid. It has altogether 24 differential segments (as compared with 13 in *Paris polyphylla*). Within, or at the end

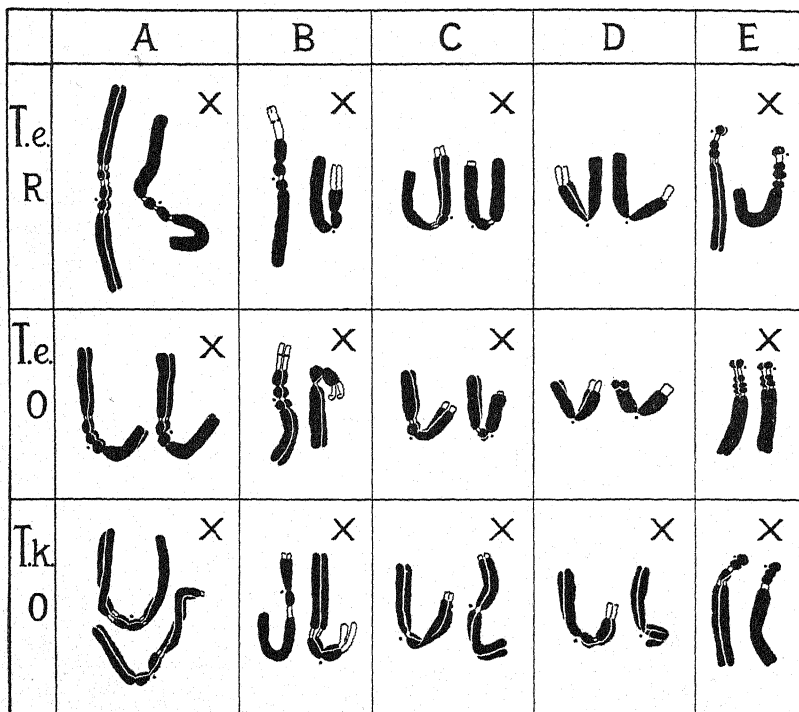


Text-fig. 6. *T. grandiflorum*, chilled chromosomes of seven plants, all with different heterochromatic segments (cf. Text-fig. 5). $\times 1000$.

194 *Nucleic Acid Starvation of Chromosomes in Trillium*

of, one of these in the *E* chromosome lies its centromere—in all other chromosomes well separated from the differential segments.

T. kamschaticum is the only Asiatic species in our list. Its chromosomes seem to correspond in shape with Haga's (1934) illustrations, which of course distinguish no differential regions. Our plant is hybrid in the structure of every chromosome: *A* and *C* in regard to terminal segments, *E* in regard to an intercalary segment and *B* and *D* in regard to



Text-fig. 7. *T. erectum*, root-tip and ovary wall, and *T. kamschaticum*, ovary wall, chilled chromosomes. Dots indicate positions of centromeres. Other constrictions are due to small intercalary segments of heterochromatin. $\times 1000$.

both. The intercalary difference in *B* may be expressed most simply as a difference in the position of the centromere, a difference to which the positions of the differential segments merely call attention.

Reviewing this evidence, we see first that this series of plants reveals structural variation in the chromosomes under natural conditions on a scale not hitherto suspected in this or any other group.¹ Only in the

¹ We are not taking to heart Jeffrey & Haertl's (1939) unconventional account of homozygous apomixis in these *Trillium* species any more than we are taking to heart Jeffrey's (1937) conventional account of their chromosome structure.

cultivated and clonal tulips perhaps can we find evidence of a comparable degree of variation and hybridity. But there the evidence is derived from inversion crossing-over (Upcott, 1937).

Secondly, we notice a regular character in the variation and hybridity. The two are of course correlated in position on any one chromosome type and in any one species. The most variable chromosomes are also the most hybrid chromosomes. But there is another property in the variation that reveals a new principle, namely its distribution. The differential chromosome types have a characteristic grade of variability. *B*, *C* and *D* are the most variable (and most hybrid). *E* is slightly less so. *A* is invariable in all except two species.

It might seem at once obvious that this grade of variability is a function of the presence of differential segments. But the objection arises

TABLE II

Position and hybridity of under-charged segments in sixteen individuals of six species of Trillium (Text-figs. 2-8)

Chromosome type ...	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	Total
Terminal segments:						
Total	1	31	24	24	0	80
Hybrid pairs	1	5	7	10	0	23
Intercalary segments:						
Total	9	9	26	9	38	91
Hybrid pairs	2	3	2	1	3	11
Total segments:						
Total	10	40	50	33	38	171
Hybrid pairs	3	8	9	11	3	34

that it is only by virtue of the differential segments that small differences in structure become visible and the correlation is purely one of observational technique. This view is contradicted by further analysis. If we classify the segments according to their position we see that the degree of hybridity is higher in the terminal segments than in the intercalary ones, although the observational test is the same in the two types. It follows that the terminal segments at least are subject to a specially high chance of variation. The proportion of hybridity is so high that losses and duplications in these segments must be assumed (as we assumed in *Paris*) to be of no immediate account.

Inertness (which is here implied) explains the difference in hybridity according to position, provided however that breakage and loss is the main cause of change. This possibility can be studied at the later stages of mitosis.

4. ANAPHASE: THE REPRODUCTIVE ERROR

Behaviour is normal throughout mitosis under normal temperature conditions in *Trillium grandiflorum* and *T. stylosum*. In the chilled preparations, however, the separation of the differential segments at anaphase is frequently abnormal.

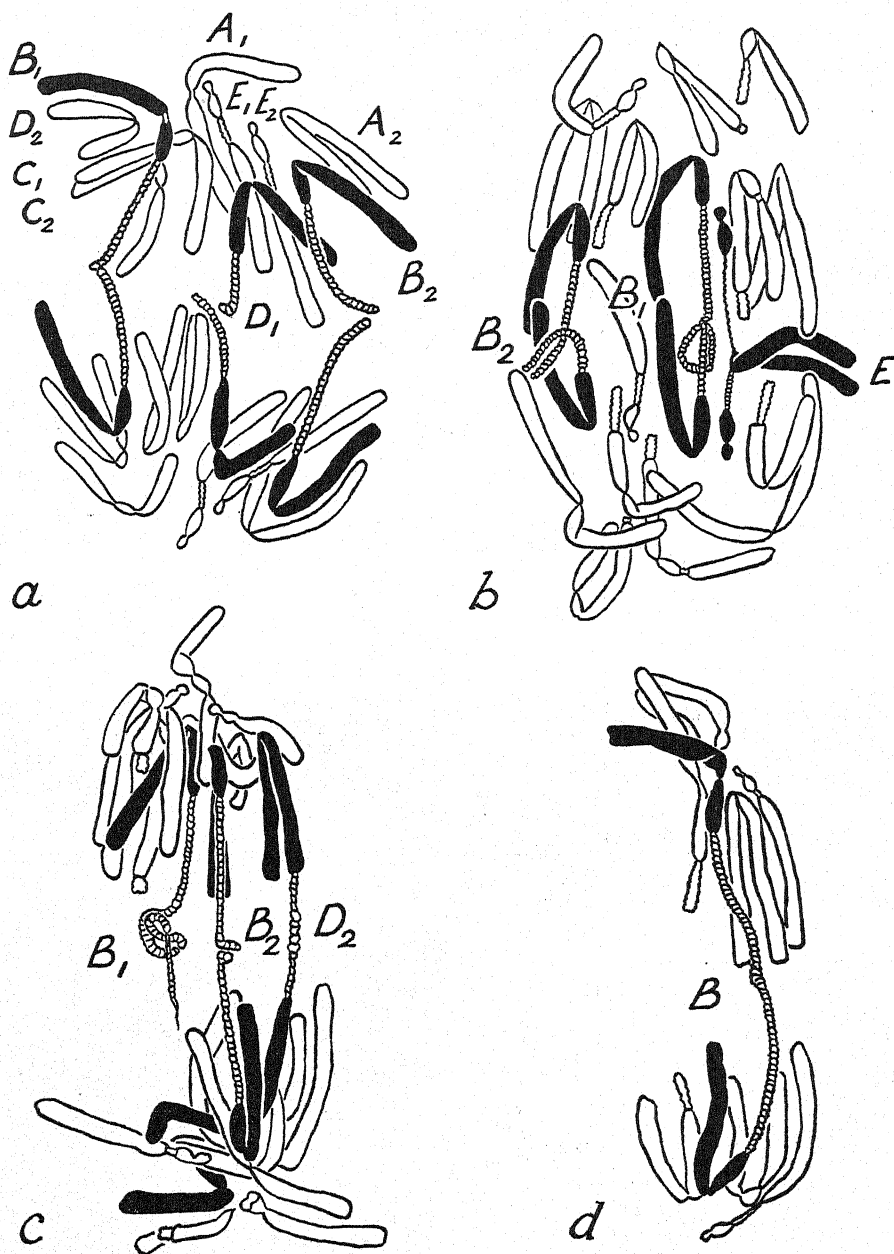
The abnormality takes two contrasted forms, intercalary adhesion, as though the internal coils were interlocked, and terminal attachment. Both are analogous to abnormalities of first anaphase of meiosis. The first resembles the Klingstedt effect (1939) where the attraction of chromatids fails to lapse on the far side of the proximal chiasma. The second resembles the bridge formation by dicentric chromatids which follows inversion crossing-over (Text-fig. 8).

This contrast is superficial. The intercalary adhesion which seems to be due to delayed separation of independent chromatids is stronger in fact than the internal cohesion of the chromatids themselves. For, as the anaphase advances and the tension of the attached chromatids increases, they stretch and are eventually broken if they are not long enough to take the strain. Moreover there are intermediate conditions. The bridge may have a median lump which presumably arises from fusion near to but not at the end (Text-fig. 9).

Both sticky chromatids and bridges are therefore the result of a union of sister chromatids intercalary or terminal in position. And when the intercalary union takes place it is frequently accompanied by a terminal union of the same two chromatids. Not only this but there is a correlation between the position of the intercalary union and the frequency of bridges. Comparing plants 3 and 7 of *T. grandiflorum* we find that the differential segments of *B* are shorter in 3 than in 7. These shorter segments stick closer to the end and also have a higher proportion of bridges. Further in plant 7 *B*₂ has a shorter differential than *B*₁ (9 μ against 16 μ). It sticks less often (4 times against 13 times). It never sticks in a cell in which *B*₁ does not also stick. And it sticks nearer the end (Table III).

It might seem that there is a certain optimum distance along the differential for sticking, and therefore an optimum length of differential for bridge formation. But on the other hand there is evidently no clear minimum, since the short intercalary *E* segments stick proportionately as often as the longer segments in *B* and *D*.

The consequences of sticking and bridge formation are of the usual kind. The chromosomes break close to the point of sticking and the distal



Text-fig. 8. Anaphases in chilled mitoses. *a*, *T. stylosum* 3; *b* and *c*, *T. grandiflorum* 7; *d*, *T. grandiflorum* 3. Lagging chromosomes as in Text-figs. 4-6. Heterochromatic segments lagging in *a* and *b*, attached at intercalary points in *b* (B_1 and E) and *c* (B_1 and B_2) and at or near the end to give a bridge in *c* (D_2) and *d* (B). *c* and *d* are incomplete. B_1 in *c* has broken. $\times 1800$.

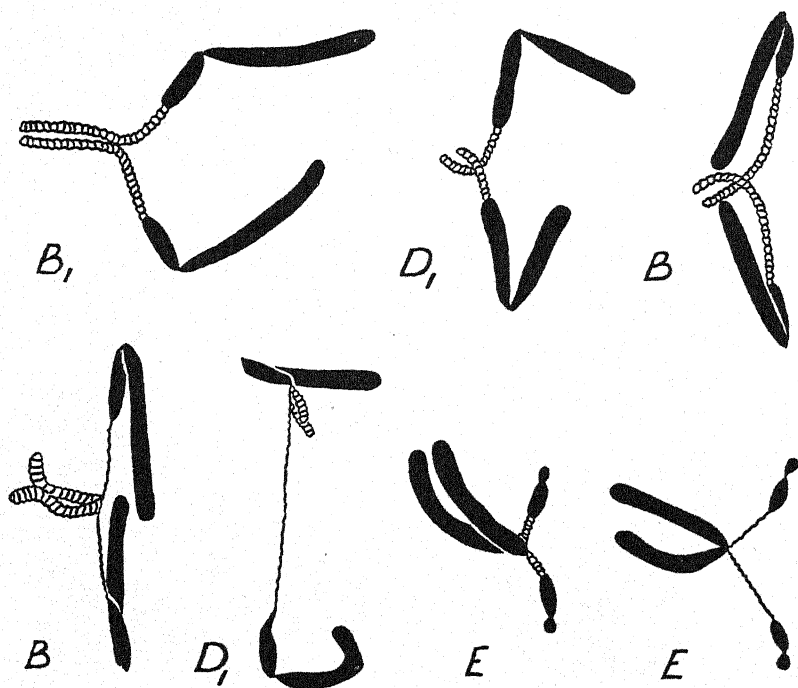
198 *Nucleic Acid Starvation of Chromosomes in Trillium*

segments are lost (Text-fig. 8). The bridges likewise mostly break, but not necessarily at the junction, so that this mishap will lead to gain for one cell equal to the loss from the other. Now seeds of *Paris* germinate at

TABLE III

Frequency and distribution of anaphase errors in T. grandiflorum and T. stylosum (metaphases illustrated in Text-figs. 4 and 6)

Plant	Total cells	Adhesion (intercalary)			Bridges (terminal) B and D	Normal cells
		B	D	E		
<i>T. grandiflorum</i> 3	47	8	—	—	20	25
<i>T. grandiflorum</i> 7	25	17	2	1	7	9
<i>T. stylosum</i> 1, 2, 3	55	18	2	2	4	35
Total	127	43	4	3	31	69



Text-fig. 9. Chilled chromosomes lagging at anaphase: above earlier stages with uncertain attachment, below later stages with certain attachment (cf. Pl. IX). The first figure is from *T. g. 7*, the rest from *T. stylosum*. $\times 1800$.

0° C. under the conditions which lead to these irregularities. We therefore need to look no further for the sources of the specially high variability in the terminal differential segments in *Paris* and *Trillium*.

5. RESTING STAGE: REVERSIBILITY OF THE REACTION

The resting nucleus in *Trillium* has been examined by Resende (1937). He finds that "Die Untersuchung der Nucleolen ist bei *Trillium* besonders schwierig, weil im Kern ausserdem viele runde Chromozentren vorkommen, die so gross wie die Nucleolen sind". He concludes that there are nucleoli in *T. longiflorum* "die in keiner Verbindung mit irgendwelchen fadenartigen Bildungen stehen", in other words some of the nucleoli arise without specific organisers on the chromosomes. Matsuura (1938) similarly finds terminal nucleoli with no trabant or constriction in *T. kamtschaticum*. Nucleoli often arise at the ends of the chromosomes and are then no doubt responsible for the appearance of extremely small trabants, which however apart from plant 1 of *T. sessile* (Text-fig. 2) are variable in number (Text-figs. 3, 5 and 6).

This variability of trabants in *Trillium* is paralleled by the variability in a secondary constriction in *Paris polyphylla* (Text-figs. 3-5 in our paper). We conclude that there are no constant secondary constrictions in normal temperature preparations of any of our species of *Paris* and *Trillium* and therefore no constant nucleolar organizers. The situation is like that arising in deficient nuclei of *Zea Mays* (McClintock, 1934).

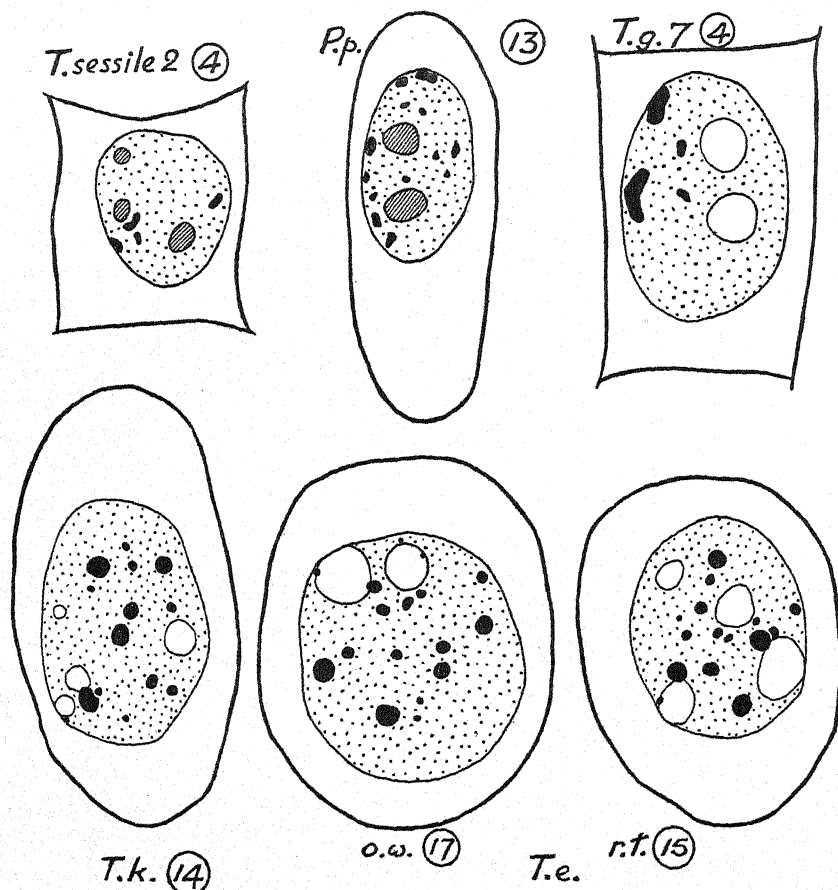
We are, however, chiefly concerned with the "chromocentres" or deeply stained parts of the chromosomes which are still recognizable in the resting nucleus. These bodies have been studied in many plants by Heitz (1932), Manton (1935) and Resende (1937), who have shown constancy in their number, form and position for the individual or the species. Our illustrations (Text-fig. 10) can now be compared with the differential segments shown at metaphase.

The chromocentres, especially in *T. erectum*, are darkly stained and highly refractive, so that the larger ones resemble metaphase chromosomes. The conditions of their fixation however compare unfavourably with those of metaphase chromosomes, and they characteristically show the bubble artefacts which imply a defective fixation at metaphase.

Thirteen chromocentres in *Paris polyphylla* correspond with its thirteen differentials. In *T. erectum*, where 24 differentials are seen, the number of chromocentres is smaller and not always accurately ascertainable. Here as elsewhere the smaller intercalary segments are not always distinguishable. Thus the four chromocentres in the resting nucleus of *T. sessile* and *T. grandiflorum* probably correspond with the four larger differentials in the metaphase chromosomes of these species. It is also possible that two centres lying close together may appear as one. The small normal inter-

200 Nucleic Acid Starvation of Chromosomes in *Trillium*

calary segment in *T. erectum* would not appear as an interruption in the resting stage and a small intercalary centre of corresponding size would certainly escape notice at metaphase as well.



Text-fig. 10. Resting stages in five species of *Paris* and *Trillium*. Specimens from root-tip and ovary wall in *T. erectum*, the rest from root-tips. *T. sessile*, normal temperature; the rest chilled. Nucleolus hatched in gentian violet preparations, blank in Feulgen preparations. Number of chromocentres in each nucleus given in ring. Compare these with number of heterochromatin segments at metaphase (Text-figs. 2-7). The large segments in *T. g. 7* are evidently due to fusion. $\times 1500$.

The sizes of the chromocentres likewise bear a general proportion to the sizes of the differentials, and this is especially clear in comparing species. For example, one could not mistake the large centres of *T. grandiflorum*, with its large terminal differentials, for the minute centres of *Paris (Kinagusa) japonica*, with its small intercalary differentials: so

small in fact that their presence is only to be noted in the light of the other evidence (Text-fig. 11; cf. Haga, 1934).

This comparison leaves no doubt that the over-nucleated chromocentres of the resting stage are in fact the under-nucleated differential segments of metaphase. They are the heterochromatic part of the chromosomes, and in revealing the differentials we have found a temperature threshold for establishing a differential nucleic acid content at metaphase such as has hitherto been known only within the resting and prophase nucleus.

The under-charged metaphase segments thus arise at low temperature from over-charged resting stage segments. But it must not be assumed from this that the reverse is true. Mitoses taking place at low temperature would not necessarily give rise to the customary over-charged segments at the following resting stage. Nor can we be sure that all phases of development in the resting nucleus continue to be normal at low temperatures. These questions are not readily answerable from root-tip preparations. Their importance will be seen later.

6. SUPPLY AND DEMAND OF NUCLEIC ACID

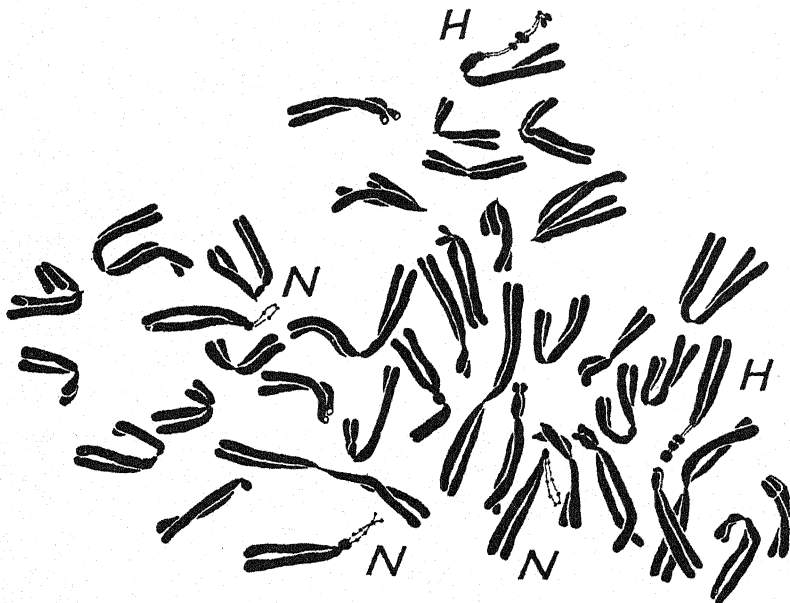
During the last 10 years several lines of research have converged to provide us with a picture of the chemical framework of chromosomes and of the cyclical changes they undergo in mitosis. To understand the functions of nucleic acid we must first examine this picture in outline.

The chromosomes are chemically and physiologically unique in combining the fibrous structure and indefinite extension of the larger inactive proteins with the activity of the smaller globular proteins. Further they undergo a cycle of reproduction, spiralization and separation which is similarly unique. This cycle is co-ordinated for all the chromosomes in a nucleus and this co-ordination is correlated with a cycle of attachment and detachment of nucleic acid to the chromomeres (cf. Caspersson *et al.* 1935). The chromomeres, which are chemically identifiable by the specificity of this action, correspond to the genes which are genetically identifiable by the specificity of their physiological action, as shown by their pairing properties and by the effects of X-ray breakage of the protein fibre.

Nucleic acid may be demonstrated equally by the ultra-violet absorption spectrum with a maximum at 2600 Å. and by the Feulgen reaction following hydrolysis (Caspersson, 1936*a*, 1937*a, b*; Hillary, 1939). During the resting stage it is present in the nucleus in smaller quantity and largely unattached to the chromosomes. Nucleic acids, having in

202 Nucleic Acid Starvation of Chromosomes in Trillium

their simplest units a molecular weight of at least 1300 (Gulland, 1938), presumably exist inside the nucleus as polymers arising from such units. During mitosis the nucleic acid increases in quantity and is attached to the protein framework of the chromosomes (Caspersson, 1937*a*). It may be digested with nuclease to leave the framework intact (Mazia & Jaeger, 1939). Nevertheless the nucleic acid does not lie on the surface of the spiralized chromosome but is bound up with the coiled thread itself



Text-fig. 11. Chilled metaphase in *Paris (Kinagusa) japonica*, $2n=40=8x$. Three chromosomes with nucleolar trabants (*N*) and two with short heterochromatic segments (*H*). Compare Haga's figures (1934) of normal temperature chromosomes. $\times 1000$.

(Caspersson, 1936*a*), the "matrix" offixation having of course no relevance to the living chromosome.

Now Astbury's measurement of the spacing of the nucleotides in sodium thymonucleate, shown by X-ray photography to be 3.34 \AA ., agrees with that of the side-chains in a fully extended polypeptide (cf. Gulland, 1938). It is therefore possible to suppose that the nucleic acid is formed through the agency of the genes arranged on the polypeptide chain and, being attached to them, serves as an agent in their reproduction, in their separation and even perhaps in their spiralization. All these possibilities arise in view of the cyclical correlation of gene reproduction and nucleic acid attachment (Caspersson & Schultz, 1938; Astbury, 1940). We may also ask ourselves whether the initiation of mitosis is

conditioned by the presence of a threshold quantity of nucleic acid (Caspersson, 1936a).

It is in relation to these chemical potentialities of the system that we have to consider the way in which departures from the regular nucleic acid cycle take place. The chromosome cycle is adjusted to have a maximum aggregate nucleic acid attachment at metaphase. It is also, with exceptions, adjusted to have a uniform increase in nucleic acid attachment for all parts of the chromosomes during prophase, ending in a uniform distribution at metaphase. It is the exceptions which concern us. In some organisms during prophase of meiosis the proximal parts of the chromosomes are over-charged, e.g. at pachytene in *Agapanthus*, at diplotene in *Zea Mays*. This spatially regular departure is presumably spatially determined by the proximity of the centromere. How important similar spatial determination may be at mitosis we do not know.

There is a second departure which is not spatially determined but is specific to the genes or groups of genes affected. These genes may be properly described as *heterochromatin* and they seem at first sight to have a great array of discordant properties which are not always rendered less discordant by the description given them by their discoverer (Heitz, 1935).¹

We see heterochromatin typically in the Y-chromosome of *Drosophila* and in the special segments of *Trillium*. These segments are over-charged in the resting stage and as a rule normally charged and indistinguishable at metaphase. Thus the chromosome cycle, although correlated with the aggregate nucleic acid attachment, is not dependent on the uniformity of all the parts. The inert B chromosomes of *Zea Mays*, we find, behave in the same way.

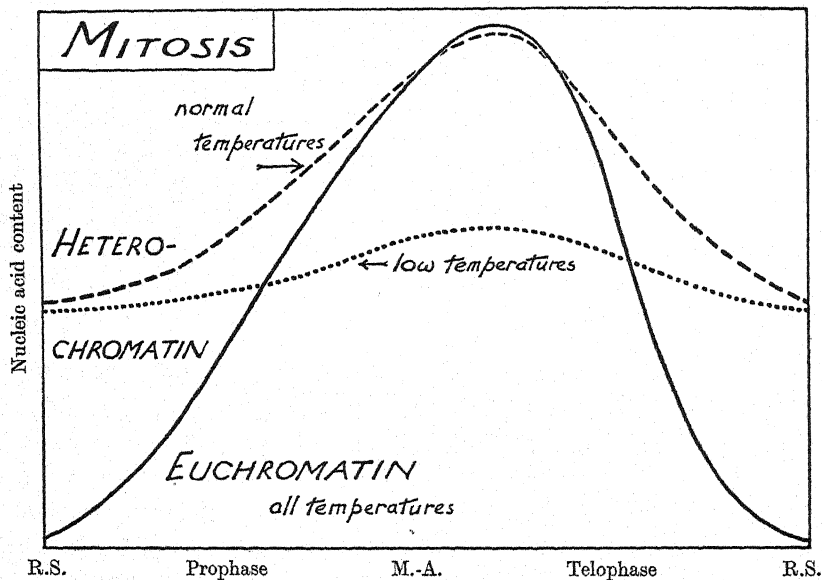
After its genetic specificity, the next important property we have to note in heterochromatin is that its departure from the normal cycle is physiologically conditioned. This is shown in the first instance genotypically. The X-chromosomes of male Orthoptera are over-charged in the resting stages of pre-meiotic mitosis. Those of females are not over-charged. It is further shown developmentally. The heterochromatin of *Drosophila*, over-charged in the mitotic resting stages, is under-charged in the salivary glands. The same reversal is found in the pre-meiotic metaphases in Orthoptera, and at meiosis in mammals. The X may be under-charged at metaphase. The same, as we see, is true of *Trillium*, and again

¹ We assume from our experience that Heitz's statement (1935, p. 409) "Durch die Feulgensche Nuklealreaktion lässt sich das Heterochromatin vom Euchromatin nicht unterscheiden" is a mistake.

204 Nucleic Acid Starvation of Chromosomes in Trillium

we have variability in the under-charging, but there we now know that conditioning is environmental and not developmental.

Conditioning and the consequent reversibility of the nucleic acid cycle of heterochromatin can now be regarded as its most significant properties. Heterochromatin is *allocyclic*. Euchromatin has a regular correlation of nucleic acid attachment and reproductive cycle. Heterochromatin either has a reduced correlation or a reversed one. It is irregular and variable in its degree of attachment. All these properties follow if we assume that heterochromatin has a lower reactivity with nucleic acid

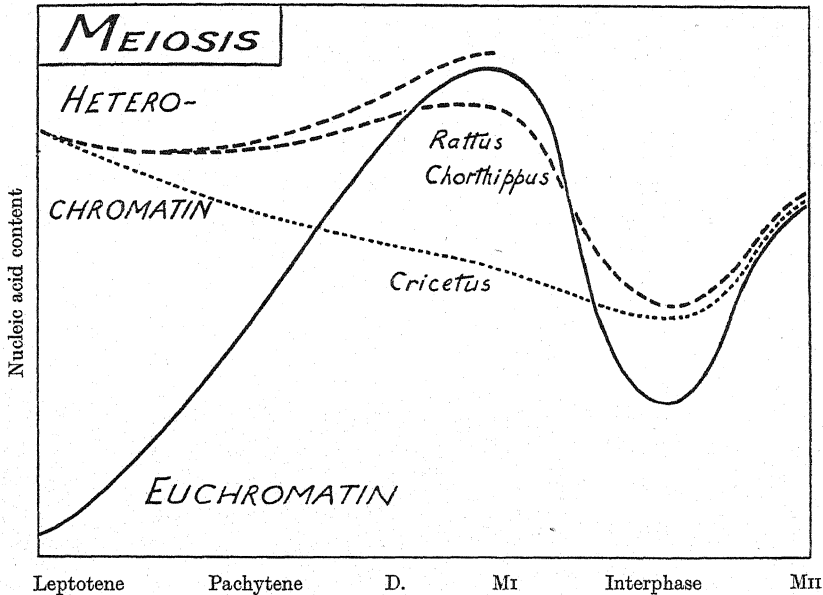


Text-fig. 12. Graph with arbitrary scale of co-ordinates to show quantitative relations of nucleic acid content of heterochromatin and euchromatin during mitosis in *Paris* and *Trillium* at normal and marginal temperatures. The heterochromatin at marginal temperatures corresponds with that in Orthoptera during rapid mitoses.

than euchromatin. Reversibility then becomes conditioning of the heterochromatin cycle by the predominant euchromatin cycle. Heterochromatin is chemically subordinate. This becomes more significant when we recall that heterochromatin is, so far as we know, genetically inert and that a recessive mutant gene in the white locus in *Drosophila* has been shown to attach less nucleic acid than its normal progenitor (Morgan *et al.* 1938).

So much may be said of the varying demand for nucleic acid in the nucleus. But supply also varies. *XXY* eggs in *Drosophila* contain more nucleic acid precursors (pyrimidine components) than the normal *XX*

(Caspersson & Schultz, 1938) and the same applies to the comparison of salivary gland nuclei in *XXY* and *XX* females. Further, when euchromatic genes are moved next to inert genes, by X-ray breakage, their nucleic acid charge is enhanced. The inert and less reactive genes of the Y therefore directly or indirectly contribute to the supply without contributing proportionately to the destruction or utilization of nucleic



Text-fig. 13. Graph corresponding to Text-fig. 12 to show nucleic acid cycles of differential segments of X-chromosomes (largely heterochromatin) at meiosis in male mammals and orthopterans. The metaphase content of nucleic acid is always variable in heterochromatin, as indicated by the interrupted alternative curve for *Rattus* and *Chorthippus*. N.B. Koller has represented the differential segment of the X in *Cricetus* as merely under-spiralized. It now seems that, not spiralization, but nucleic acid charge is at fault.

acid. And the relation between supply and demand is so direct or so rapid as to be locally variable within one nucleus.

When the *Trillium* experiments are examined in the light of a variable supply and demand of nucleic acid they begin to be intelligible. At normal temperatures the supply of nucleic acid is enough to provide an apparently equal quantity for all parts of the chromosomes. At low temperatures the supply is reduced. The chemically, and as we suppose genetically, inactive parts have to go short during the middle of the mitotic cycle when the demand is highest. The cycle of the heterochro-

206 *Nucleic Acid Starvation of Chromosomes in Trillium*

matin is flattened out just as it is under conditions of excessive strain in animals—in the rapid divisions leading to meiosis (White, 1937, 1940).

Why should the supply fail so much more obviously in *Paris* and *Trillium* than elsewhere? The impermeability of the nuclear membrane to nucleic acid at the low temperature might be affecting an intermediate step. There is, however, a possibility of a fundamental difference in nucleic acid demand. The *Paris* and *Trillium* group is remarkable for giving a stronger Feulgen reaction at metaphase of mitosis than any other plants that we are familiar with. This is not due to the chromosomes being the largest in flowering plants. For in *Fritillaria*, *Tulipa*, *Hyacinthus*, *Allium* and *Tradescantia* the chromosomes are almost as large and give a weaker reaction using our exactly standardized treatment.

This comparison depends on the combination of a simple visual test with microphotography. It is supported by the special properties of *T. erectum*. This species seems to have the strongest reaction of any at metaphase. It is therefore significant that in the resting stage the heterochromatin is stained so excessively as to appear in the form of large relatively opaque globules. This species has perhaps a greater supply of nucleic acid both in the resting stage and at metaphase.

Now the contrast between heterochromatin and other segments remains as strong as ever at metaphase in *T. erectum*. It seems therefore that the higher normal demand for nucleic acid, combined with the lower supply at lower temperatures, is what makes it possible to reach a threshold for differential attachment of nucleic acid at metaphase in *Trillium* and *Paris*.

7. ALLOCYCLY AND GENE REPRODUCTION

The chemical structure and cyclical changes of nucleic acid suggest, as we have seen, that it is an agent of gene reproduction. The failure of separation of the under-charged segments in *Trillium* supports this view, although it does not yet enable us to say whether excess of nucleic acid during the resting stage has interfered with the primary process of reproduction, or whether a deficiency during the prophase has interfered with some secondary process of separation.

There is the same difficulty in interpreting the observations of Caspersson & Schultz (1938). They find that the upset of the nucleic acid cycle in active genes translocated to heterochromatic segments goes with an irregularity in the reproduction of these genes and thus leads to somatic mutation and the appearance of mottling. This irregularity is decreased by an increase in the nucleic acid content of the egg, as in the

eggs of the *XXY* females already referred to. The local demand and total supply of nucleic acid are therefore both concerned in the reproduction of the gene just as in the allocyclic segments of *Trillium*. And their experiments are parallel with ours. They substitute a genetic control for an external one.

It is now therefore worth while finding out how far nucleic acid abnormalities may be connected with abnormalities in the timing and regularity of gene division elsewhere.

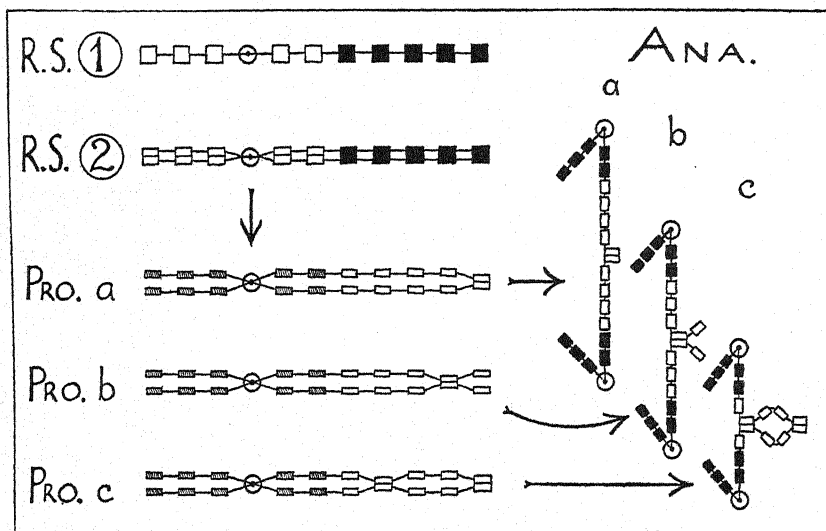
We find that reproduction takes place earlier at meiosis when there is an apparent excess of nucleic acid. This is true of sex and *M*-chromosomes in the Heteroptera (Darlington, 1939). It is true also of the two *X*-chromosomes in the male cells of hermaphrodite *Rhabditis* (Darlington, 1937). These are seemingly the converse of the conditions of non-division in under-charged segments in *Trillium*. They are also perhaps the converse of the conditions of the delayed division cycle of the centrogenes, with a lower nucleic acid content, relative to the common genes.

There are other examples where an excess of nucleic acid goes with non-specificity of pairing as in the "*B*" chromosomes of maize, or complete immobilization as in the pachytene *X* of Orthoptera and the first metaphase *Y* in "sex-ratio" *Drosophila* (Sturtevant & Dobzhansky, 1936). But these effects may well be complicated by the adsorption of accessory materials other than nucleic acid (cf. Bauer, 1933). Again many errors of reproduction have not been related to the nucleic acid cycle and might well repay examination from this point of view. Such are the sticky chromosomes of a *Chorthippus* hybrid (Klingstedt, 1939) and of "sticky" maize (Beadle, 1932). Nor is it easy to tell (without temperature experiments) whether the double division of the *X*-chromosome in "sex-ratio" males of *Drosophila* is the cause or the consequence of an increased nucleic acid content. This double division of *X* in "sex-ratio", which is an abnormality of reproduction, however, goes with a failure of separation of parts of chromatids at anaphase not unlike that in *Trillium*. It also goes with an obvious nucleic acid abnormality of the *Y*. A similar failure of separation of the meiotic chromatids occurs in the same *Chorthippus* which has bridge formation at earlier mitoses.

Other errors of reproduction which cannot yet be related to nucleic acid metabolism are those responsible for spontaneous bridges in pollen grains (Upcott, 1937*a*; Giles, 1940) and over-ripe pollen tubes (Barber, 1938). Such spontaneous changes are of widespread occurrence. Our experience indicates that the mutations found on the germination of old seeds are not due to spontaneous breaks during storage but to reproductive

abnormalities at the mitoses after storage. They would then be placed in the same class as those in the pollen. And with these too may be placed the great array of controlled adaptive errors classed as chromosome elimination and diminution.

The reproductive errors in *Trillium* thus provide a connecting link between instances of a proved relationship of reproduction and the nucleic acid cycle on the one hand and a wide range of unplaced errors on the other. Of these latter some are genotypically and others developmentally



Text-fig. 14. Diagram showing possible nature of sticking or non-reproduction of intercalary and terminal genes to give three kinds of abnormalities (*a*, *b*, *c*) seen at anaphase in *Trillium*. Reversal of nucleic acid relationship of heterochromatin between resting stage (*R.S.*) and anaphase.

or environmentally conditioned. The possibility of modifying the nucleic acid supply by temperature brings these within reach of experiment.

There are two other directions in which the present work suggests new interpretations. The first is concerned with mutability. The errors of reproduction of our under-charged segments suggest an explanation of the observation of Kaufmann (1939) that inert segments of *Drosophila* contain most repeat regions due to previous breakage and reduplication. They agree in showing that the inertness is the cause of the reduplication, not the result of it. And they indicate one of the reasons why inert genes are liable to occur in large blocks and not scattered at random among the active genes. The value of these blocks may be that they serve to regulate the crossing-over frequency in active regions.

But our temperature threshold has a special implication. If the nucleic acid content characteristic of heterochromatin is connected with this mutability, then the sharp changes occurring in nucleic acid distribution at a critical temperature would lead us to expect a related sharp change in mutability at such a temperature. The temperature coefficient of spontaneous and induced mutation would then bear no simple relationship with that of chemical reactions. This expectation is borne out by Kerkis's observations (1939) on spontaneous mutation at -5°C . and by Sax's X-ray experiments (1940).

The second direction of enquiry concerns chromosome size. We know that size can be controlled in many organisms environmentally, developmentally or genotypically. In all these ways the effect is proportionate and uniform in all parts and dimensions. Sometimes a deficiency in the supply of some necessary material seems to be the agent of a reduction in size. For example, in rapid cleavage divisions chromosome size is sometimes reduced. One example of this proportionate effect is found in *Trillium kamtschaticum* where the controlling agent is temperature (Matsuura, 1937). Now in our experiments the bulk of the heterochromatin segments is reduced when they are under-charged with nucleic acid as a result of low temperature treatment. But the change does not apply to the whole chromosome and it is not compatible with regular reproduction. Nucleic acid metabolism may then be concerned in some way with changes in chromosome size generally, but the mechanism cannot be the same as in our experiments.

Finally, with regard to spiralization, the heterochromatin segments are the same length when under-charged as when normally charged. We can therefore give a surprising answer to our earlier question: nucleic acid seems to have no necessary relation to spiralization.

8. SUMMARY

1. Those parts of the chromosomes which have the inherent property of failing to maintain the maximum nucleic acid cycle at mitosis are defined as heterochromatin.

2. The Feulgen reaction shows such specific segments, over-charged with nucleic acid, in the resting nuclei of *Paris* and *Trillium* species.

3. In metaphase chromosomes at normal temperatures these segments have the standard charge of the whole complement. But when mitosis begins at a low temperature they reach metaphase under-charged. The chromatids are then reduced to half the standard diameter but remain the standard length.

210 *Nucleic Acid Starvation of Chromosomes in Trillium*

4. Species and individuals differ in the number, size and position of the segments. They number from one to four per chromosome and may be terminal or intercalary. Short intercalary segments look like nucleolar constrictions. These however do not regularly occur in *Trillium*. There are thus in the same species constrictions without nucleoli and nucleoli without constrictions.

5. Of eighty pairs of chromosomes in sixteen individuals of six species of *Trillium*, sixty-five pairs had visible heterochromatic segments in one or both partners and of these thirty-five were hybrid pairs as shown by the disposition of the heterochromatic segments.

6. This high variability is largely due to loss and duplication of terminal segments, which must therefore be inert or sub-inert like heterochromatin in *Drosophila*.

7. The high frequency of these changes is due to the sister chromatids in the special segments failing to separate at anaphase and forming bridges at telophase. This reproductive error occurs only in chilled chromosomes and in the segments deficient in nucleic acid. It shows the activity of nucleic acid in gene reproduction also inferred elsewhere. The temperature threshold for nuclear division is below that for gene division.

8. The under-charging at metaphase arises only at marginal temperatures and seems to be conditioned by (a) a lower supply of nucleic acid in the nucleus, and (b) a lower competitive strength or demand of the inert genes for nucleic acid in the presence of an insufficient supply.

9. This view is supported by genotypically conditioned allocycly, e.g. in sex chromosomes which likewise show variation subject to environmental and developmental changes and flattening or reversal of the nucleic acid cycle.

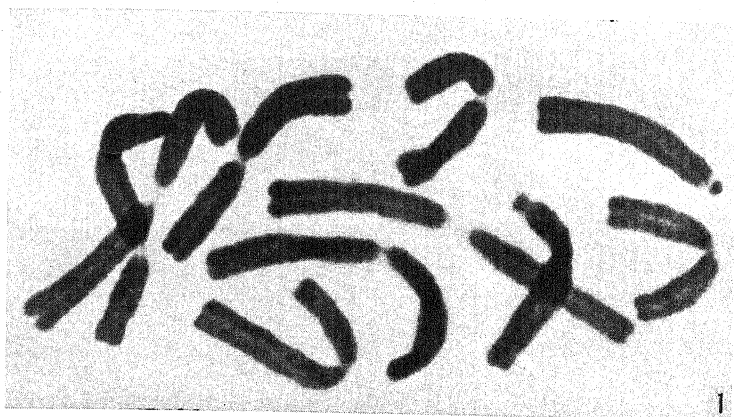
REFERENCES

- ASTBURY, W. T. (1940). "Protein and virus studies in relation to the problem of the gene." *Proc. 7th Int. Cong. Genet. Edinburgh*, 1939.
- BARBER, H. N. (1938). "Delayed mitosis and chromosome fusion." *Nature, Lond.*, **141**, 80.
- BAUER, H. (1933). "Die wachsenden Oocytenkerne einiger Insekten in ihrem Verhalten zur Nuklealfärbung." *Z. Zellforsch.* **18**, 254-98.
- BEADLE, G. W. (1932). "A gene for sticky chromosomes in *Zea Mays*." *Z. indukt. Abstamm.- u. Vererb. Lehre*, **63**, 195-217.
- BERNAL, J. D. (1939). "Structure of proteins." *Nature, Lond.*, **143**, 663-5.
- CASPERSSON, T. (1936a). "Über den chemischen Aufbau der Strukturen des Zellkernes." *Skand. Arch. Physiol.* **73**, Supp. 8.
- (1936b). "Die Untersuchung der Nukleinsäureverteilung im Zellkern." *Z. wiss. Mikr.* **53**, 413-19.

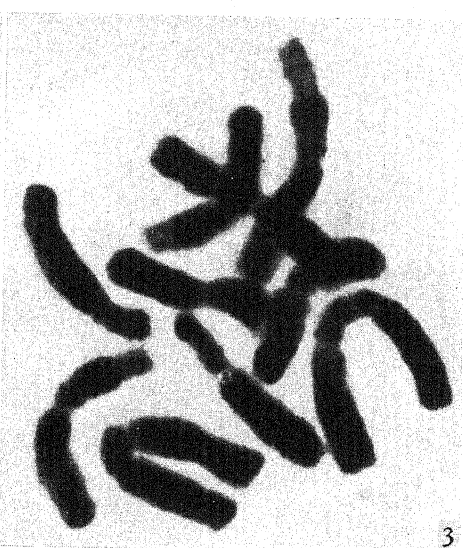
- CASPERSSON, T. (1937a). "Exposé sur la répartition des acides nucléiques dans le noyau cellulaire." *Bull. Histol. appl.* **14**, 33-43.
- (1937b). "Über den chemischen Aufbau der Strukturen des Zellkernes." (Summary.) *Protoplasma*, **27**, 463-7.
- CASPERSSON, T., HAMMARSTEN, E. & HAMMARSTEN, H. (1935). "Interactions of proteins and nucleic acid." *Trans. Faraday Soc.* **31**, 367-89.
- CASPERSSON, T. & SCHULTZ, J. (1938). "Nucleic acid metabolism of the chromosomes in relation to gene reproduction." *Nature, Lond.*, **142**, 294-5.
- DARLINGTON, C. D. (1936). "Crossing-over and its mechanical relationships in *Chorthippus* and *Stauroderus*." *J. Genet.* **33**, 465-500.
- (1937). *Recent Advances in Cytology*, 2nd ed. London: Churchill.
- (1939). "The genetical and mechanical properties of the sex chromosomes. V. *Cimex* and the Heteroptera." *J. Genet.* **39**, 101-37.
- (1940). "The prime variables of meiosis." *Biol. Rev.* **15**.
- DARLINGTON, C. D. & LA COUR, L. (1938). "Differential reactivity of the chromosomes." *Ann. Bot., Lond.*, N.S. **2**, 615-25.
- ELLENHORN, J. (1934). "Zytologische Studie über die genetisch bedeutsamen Kernstrukturen." *Z. Zellforsch.* **21**, 24-41.
- GEITLER, L. v. (1938). "Über das Wachstum von Chromozentrenkernen und zweierlei Heterochromatin bei Blütenpflanzen." *Z. Zellforsch.* **28**, 133-53.
- GILES, N. (1940). "Spontaneous chromosome aberrations in *Tradescantia*." *Genetics*, **25**, 16-40.
- GOTOH, K. (1933). "Karyologische Studien an *Paris* und *Trillium*." *Jap. J. Genet.* **8**, 197-203.
- GULLAND, J. M. (1938). "Nucleic acids." *J. chem. Soc.* pp. 1722-34.
- HAGA, T. (1934). "The comparative morphology of the chromosome complement in the tribe *Parideae*." *J. Fac. Sci. Hokkaido Univ.* v, **3**, 1-32.
- (1937a). "Chromosome complement of *Kinagusa japonica* with special reference to its origin and behaviour." *Cytologia*, **8**, 137-41.
- (1937b). "Genom and polyploidy in the genus *Trillium*. I. Chromosome affinity between the genoms." *Jap. J. Genet.* **13**, 135-45.
- (1937c). "Karyotypic polymorphism in *Paris hexaphylla* Cham., with special reference to its origin and to the meiotic chromosome behaviour." *Cytologia, Tokyo, Fujii Jub. Vol.* pp. 681-700.
- HEITZ, E. (1932). "Die Herkunft der Chromozentren." *Planta*, **18**, 571-636.
- (1935). "Chromosomenstruktur und Gene." *Z. indukt. Abstamm.- u. Vererb.-Lehre*, **70**, 402-47.
- HILLARY, B. B. (1939). "The use of the Feulgen reaction in cytology. I." *Bot. Gaz.* **101**, 276-300.
- JEFFREY, E. C. (1937). "The somatic chromosomes of *Trillium*." *Cytologia, Tokyo, Fujii Jub. Vol.* 857-66.
- JEFFREY, E. C. & HAERTL, E. J. (1939). "The product of unfertilized seeds in *Trillium*." *Science*, **90**, 81-2.
- KAUFMANN, B. P. (1939). "Distribution of induced breaks along the X-chromosome of *Drosophila melanogaster*." *Proc. nat. Acad. Sci., Wash.*, **25**, 571-7.
- KERKIS, J. (1939). "Effect of temperature below 0° upon the process of mutation and some considerations on the causes of spontaneous mutation." *C.R. Acad. Sci. U.R.S.S.* **24**, 386-8.

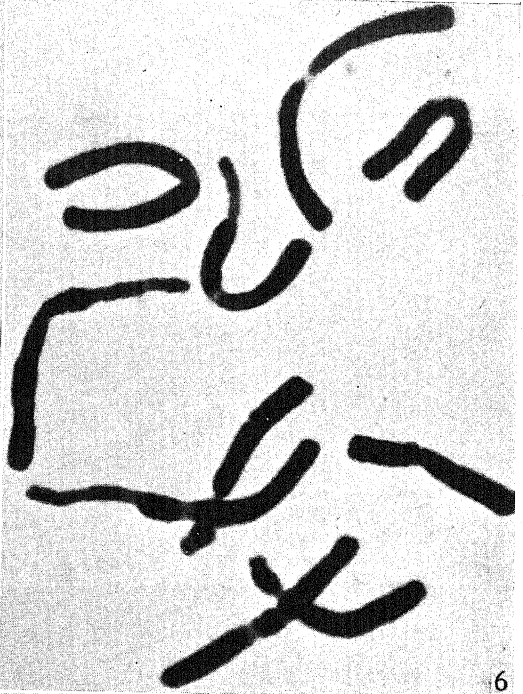
212 *Nucleic Acid Starvation of Chromosomes in Trillium*

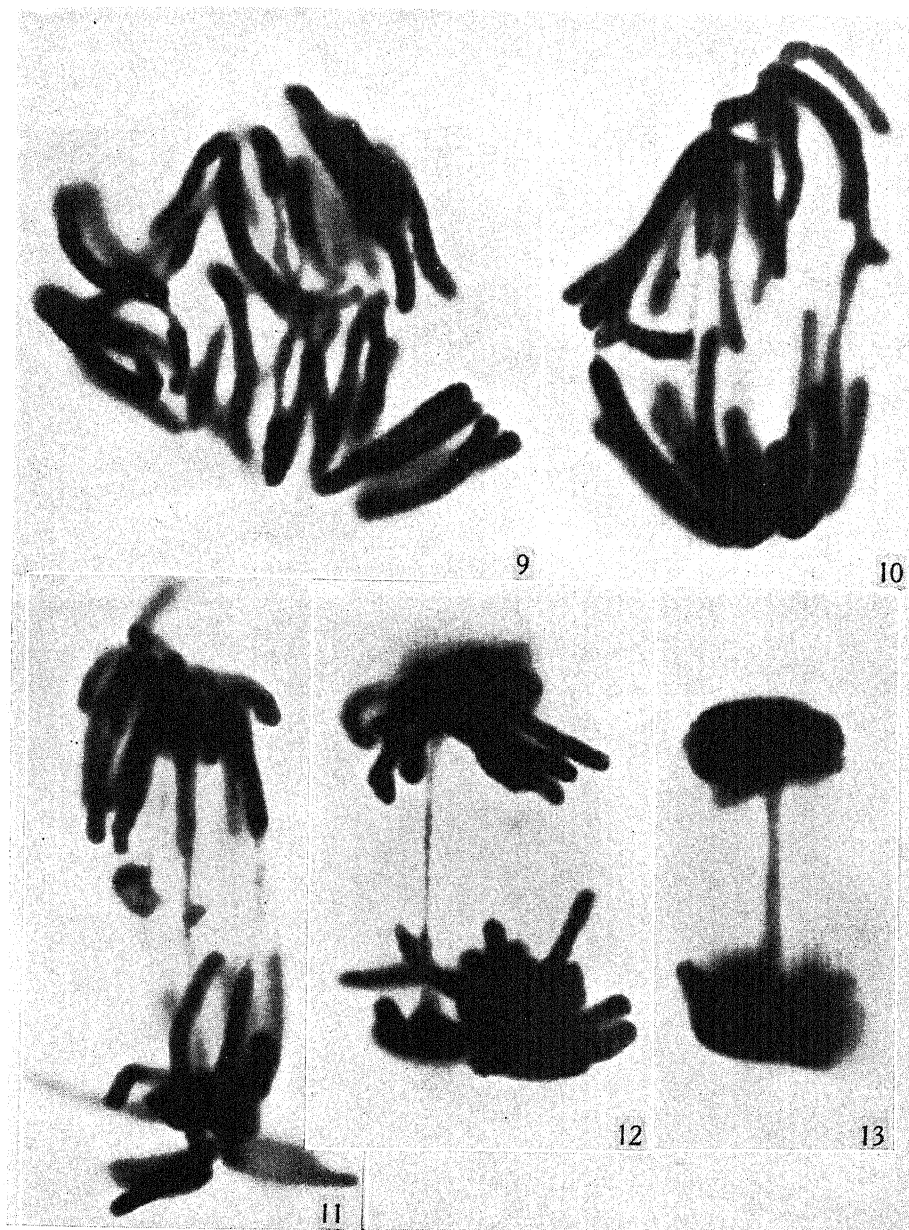
- KLINGSTEDT, H. (1939). "Taxonomic and cytological studies on grasshopper hybrids. I. Morphology and spermatogenesis of *Chorthippus bicolor* Charp. x *Ch. biguttulus* L." *J. Genet.* **37**, 389-420.
- KOLLER, P. C. (1938). "The genetical and mechanical properties of the sex-chromosomes. IV. The golden hamster." *J. Genet.* **36**, 177-95.
- KOLLER, P. C. & DARLINGTON, C. D. (1934). "The genetical and mechanical properties of the sex-chromosomes. I. *Rattus norvegicus* ♂." *J. Genet.* **29**, 159-73.
- LA COUR, L. (1937). "Improvements in plant cytological technique." *Bot. Rev.* **5**, 241-58.
- LEVAN, A. (1938). "The effect of colchicine on root mitoses in *Allium*." *Hereditas, Lund.*, **24**, 471-86.
- MCCLEINTOCK, B. (1934). "The relation of a particular chromosomal element to the development of the nucleoli in *Zea Mays*." *Z. Zellforsch.* **21**, 294-328.
- MANTON, I. (1935). "Some new evidence on the physical nature of plant nuclei from intra-specific polyploids." *Proc. roy. Soc. B*, **118**, 522-47.
- MATSUURA, H. (1937). "Chromosome studies on *Trillium kamschaticum* Pall. V." *Cytologie, Fujii Jub. Vol.* pp. 20-34.
- (1938). "Chromosome studies on *Trillium kamschaticum* Pall. VI. On the nucleolus-chromosome relationship." *Cytologia, Tokyo*, **9**, 55-77.
- MAZIA, D. & JAEGER, L. (1939). "Nuclease action, protease action and histochemical tests on salivary chromosomes of *Drosophila*." *Proc. nat. Acad. Sci., Wash.*, **25**, 456-61.
- MOFFETT, A. A. (1936). "The cytology of *Lachenalia*." *Cytologia, Tokyo*, **7**, 490-98.
- MORGAN, T. H., BRIDGES, C. B. & SCHULTZ, J. (1935, 1936, 1937, 1938). *Yearb. Carneg. Instn.*, **34**, **35**, **36**.
- RESENDE, F. (1937). "Über die Ubiquität der SAT-Chromosomen bei den Blütenpflanzen." *Planta*, **26**, 757-807.
- SAX, K. (1940). "An analysis of X-ray induced chromosomal aberrations in *Tradescantia*." *Genetics*, **25**, 41-68.
- STURTEVANT, A. H. & DOBZHANSKY, TH. (1936). "Geographical distribution and cytology of 'sex ratio' in *Drosophila pseudoobscura* and related species." *Genetics*, **21**, 473-90.
- UPCOTT, M. B. (1937a). "Spontaneous chromosome changes in pollen grains." *Nature, Lond.*, **139**, 153.
- (1937b). "The genetic structure of *Tulipa*. II. Structural hybridity." *J. Genet.* **34**, 339-98.
- WARREN, H. E. (1937). "Cytology of the Pacific Coast *Trilliums*." *Amer. J. Bot.* **24**, 376-83.
- WHITE, M. J. D. (1937). "The effect of X-rays on the first meiotic division in three species of Orthoptera." *Proc. roy. Soc. B*, **124**, 183-96.
- (1940). "The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure." *J. Genet.* **40**, 67-82.



Micrograph 2 shows a group of chromosomes, likely from a single cell, arranged in a somewhat circular pattern. The chromosomes exhibit distinct banding patterns, characteristic of G-banding. A small number '2' is visible in the bottom right corner of the image.







EXPLANATION OF PLATES VII—IX

Microphotographs (for which we are indebted to Mr H. C. Osterstock) of Feulgen smears of mitotic chromosomes in *Trillium* species illustrated in text-figures.

PLATE VII

Metaphases, $\times 1700$.

Fig. 1. *T. sessile*, normal temperature, root-tip.

Figs. 2, 3. *T. erectum*, chilled, ovary wall.

Fig. 4. *T. grandiflorum* 1, chilled, root-tip.

PLATE VIII

Metaphases and anaphases, $\times 1500$; fig. 5, $\times 1700$.

Figs. 5, 6. *T. stylosum*, plants 4 and 3.

Fig. 7. *T. grandiflorum* 7, anaphase (cf. Text-fig. 9).

Fig. 8. *T. stylosum* 3 (cf. Text-fig. 8).

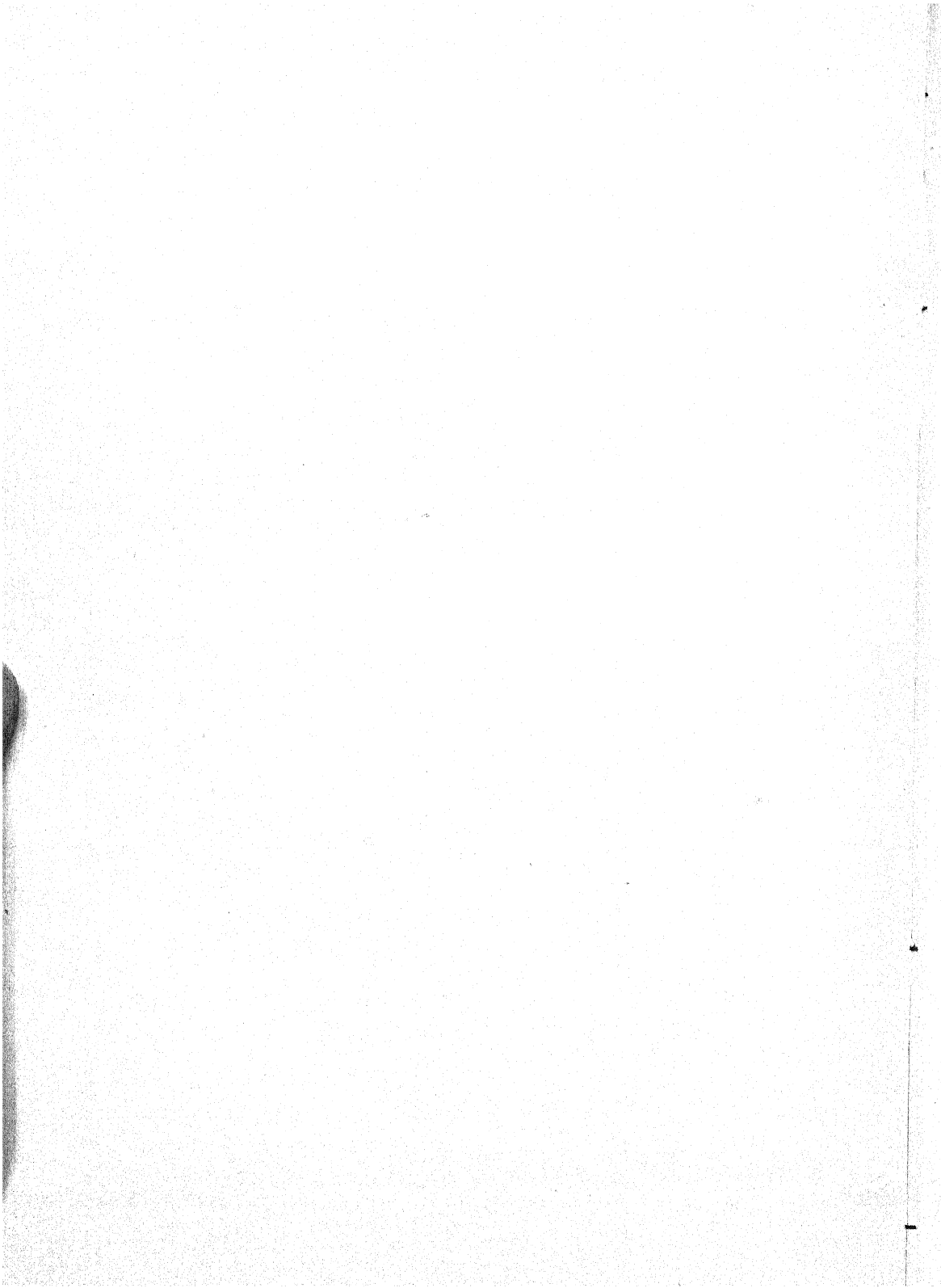
PLATE IX

Anaphases, $\times 1500$.

Figs. 9, 10 and 12. *T. stylosum* 2 (cf. Text-fig. 9).

Fig. 11. *T. grandiflorum* 7 (cf. Text-fig. 8).

Fig. 13. *T. grandiflorum* 3.



GENETICAL STUDIES ON THE PATTERN TYPES OF THE LADY-BIRD BEETLE, *HARMONIA* *AXYRIDIS* PALLAS

By YASUSI HOSINO

Zoological Institute, Kyoto Imperial University

(With Twenty-nine Text-figures)

INTRODUCTION

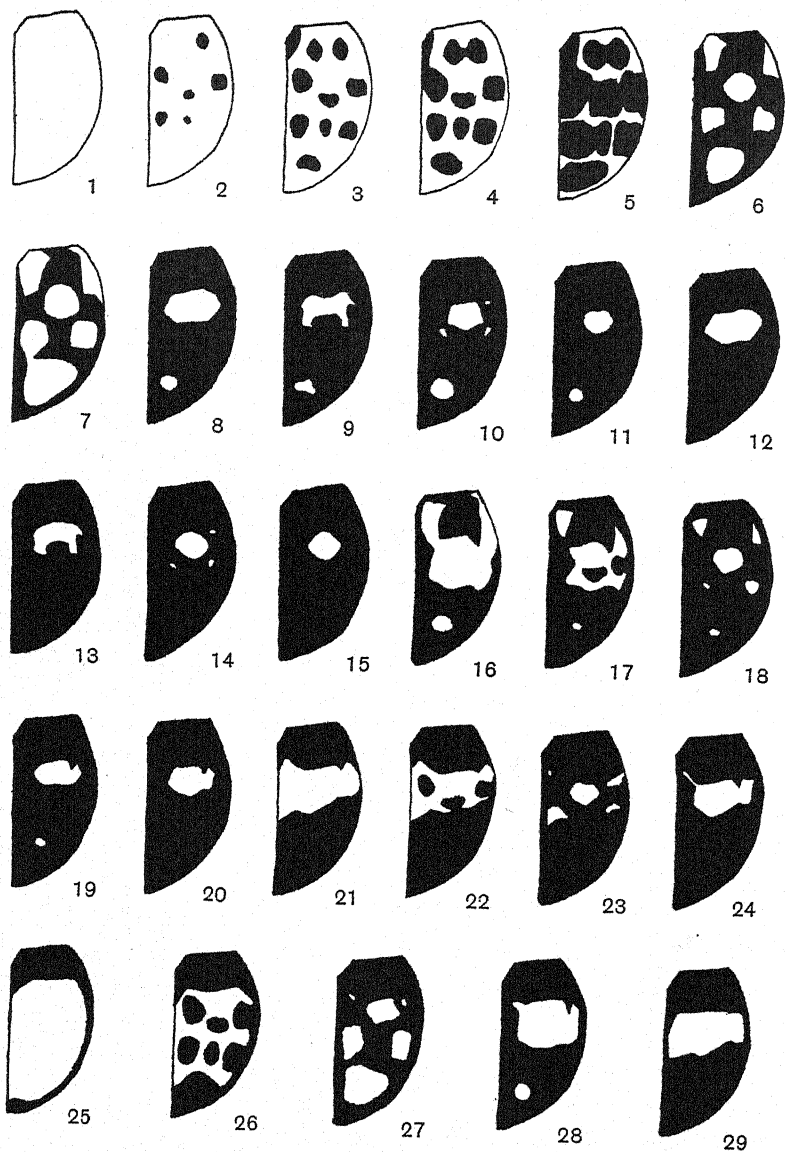
THE lady-bird beetle, *Harmonia axyridis* Pallas, is well known for the great variability of its elytral, as well as thoracic, pattern (Figs. 1-29). The ground colour of the elytra may be either orange yellow (Figs. 1-5) or black (Figs. 6-29). In the former case there are commonly nineteen black spots arranged as shown in Fig. 3 (var. *succinea*). The spots are often enlarged to become confluent with one another (Figs. 4, 5), or they diminish in size and the smaller ones disappear (Fig. 2); in the extreme case of the latter tendency the elytra become uniformly orange without any trace of spots (Fig. 1). The size of the spots in this type is apparently correlated with the amount of humidity and the degree of temperature in environment: in hot and dry climate they become smaller, while in cold and humid climate they are enlarged (cf. Dobzhansky, 1924, 1933).

Among the types with the black ground, the one in Figs. 6, 7 has six spots of orange colour on each side (var. *axyridis*). Other common types are the one with two spots on each side (var. *spectabilis*), and that with a single spot (var. *conspicua*). Those shown in Figs. 16, 17, 21, 22 and 25, 26, belong to rarer types.

Tan & Li (1932-3, 1934) have studied the heredity of these commoner types. They found that *succinea* is recessive to any other type, while *aulica*, *spectabilis* and *conspicua* are due to dominant genes independent of one another.

I have been working on the same problem since 1932. The results have confirmed Tan & Li's findings partly, but are at variance with them in an important point, namely, that the genes of all the types undoubtedly form multiple allelomorphous series, instead of being independent of one another. Though the genes for *axyridis*, *spectabilis* and *conspicua* are largely dominant to the gene for *succinea*, yet the heterozygotes can be distinguished from homozygotes by slight but distinctive features.

This paper deals with the facts which seem to be fairly firmly established by the data so far obtained. They include the heredity of all the



5 mm.

Figs. 1-29

1-5, *succinea* (pp); 6, *axyridis* (P^{AP}A, P^{AP}p); 7, *axyridis* (P^{AP}p); 8, *spectabilis* (P^{SP}SP, P^{SP}SP^{AN}, P^{SP}p); 9, *spectabilis* (P^{SP}p); 10, 11, heterozygote of *spectabilis* and *axyridis* (P^{SP}PA); 12, *conspicua* (P^{CP}CP, P^{CP}PS, P^{CP}PT, P^{CP}PA, P^{CP}PS, P^{CP}p); 13, *conspicua* (P^{CP}p); 14, 15, heterozygote of *conspicua* and *axyridis* (P^{CP}PA); 16, *forficula* (P^{FP}PF, P^{FP}p); 17, *forficula* (P^{FP}p); 18, heterozygote of *axyridis* and *forficula* (P^{AP}PF); 19, heterozygote of *spectabilis* and *forficula* (P^{SP}PF); 20, heterozygote of *conspicua* and *forficula* (P^{CP}PF); 21, *transversifascia* (P^{TP}PT, P^{TP}p); 22, *transversifascia* (P^{TP}p); 23, heterozygote of *transversifascia* and *axyridis* (P^{TP}PA); 24, heterozygote of *transversifascia* and *forficula* (P^{TP}PF); 25, *aulica* (P^{AP}PA, P^{AP}p); 26, *aulica* (P^{AP}p); 27, heterozygote of *axyridis* and *aulica* (P^{AP}PA); 28, heterozygote of *forficula* and *aulica* (P^{FP}PA); 29, heterozygote of *transversifascia* and *aulica* (P^{TP}PA).

patterns dealt with in Tan & Li's paper, besides three types which were not treated by them. Further data will be reserved for later papers.

The material was derived from individuals collected in the field in the vicinity of Tokyo. The larvae were reared in glass tubes or in Petri dishes. Various kinds of aphids obtained from the field served as food. Certain kinds of aphids are unsuitable for the purpose, apparently being poisonous to the larvae.

EXPERIMENTAL DATA

Succinea

When *succinea* is bred *inter se*, the progeny is all *succinea*, with no other type (Table I). This shows that all *succinea* individuals are homozygous for the gene.

TABLE I

Succinea × *succinea*

Cross no.	Parents	Progeny <i>succinea</i>		
		♀	♂	Total
1	pp pp	65	47	112

Axyridis

The mating *axyridis* × *axyridis* sometimes gives only *axyridis* in F_1 (Table II, no. 2), but at other times it throws *succinea* also in a nearly 3 : 1 ratio (no. 3). The backcross yields *axyridis* and *succinea* in approximately equal frequency (no. 4). Among the individuals of *axyridis*, the heterozygotes often can be distinguished from the homozygotes in that the spots are confluent with one another (Fig. 7). However, the heterozygote may show the same appearance as the homozygote (Fig. 6).

Consequently, it is clear that *axyridis* behaves as a simple dominant to *succinea*. P^A will be used as the symbol for *axyridis* and p that for *succinea*.

TABLE II

Nos. 2, 3, *axyridis* × *axyridis*; no. 4, *axyridis* × *succinea*

Cross no.	Parents	Progeny						
		<i>axyridis</i>			<i>succinea</i>			Total
		♀	♂	Total	♀	♂	Total	
2	$P^A P^A$ $P^A P^A$	19	14	33	—	—	—	—
3	$P^A p$ $P^A p$	28	25	53	11	8	19	72
Ratio (cross 3)		2.944			1.056			4.000
4	$P^A p$ pp	18	13	31	16	9	25	56
Ratio (cross 4)		1.107			0.893			2.000

Spectabilis

Spectabilis × *spectabilis* gives exclusively *spectabilis* in some cultures (Table III, no. 5), while in others it gives *spectabilis* and *succinea* in a ratio approximately 3 : 1 (no. 6). Among the individuals of *spectabilis*, the heterozygotes often can be distinguished from the homozygotes in that the anterior spot has a concavity on both the postero-lateral and posterior sides (Fig. 9). The concavity, however, is missing in some cases, and the heterozygote may show the same appearance as the homozygote

TABLE III
Spectabilis × *spectabilis*

Cross no.	Parents	Progeny						
		<i>spectabilis</i>			<i>succinea</i>			Total
		♀	♂	Total	♀	♂	Total	
5	P ^s P ^s P ^s P ^s	63	50	113	—	—	—	—
6	P ^s P P ^s P	82	72	154	29	27	56	210
Ratio (cross 6)				2.933	1.065			4.000

TABLE IV

Nos. 7, 8, *spectabilis* × *axyridis*; no. 9, *heterozygote of spectabilis and axyridis* × *heterozygote of spectabilis and axyridis*

Cross no.	Parents	Progeny									
		<i>spectabilis</i>			Heterozygote of <i>spectabilis</i> and <i>axyridis</i>			<i>axyridis</i>			Total
		♀	♂	Total	♀	♂	Total	♀	♂	Total	
7	P ^s P ^s P ^A P ^A	—	—	—	8	13	21	—	—	—	—
8	P ^s P P ^A P ^A	—	—	—	18	13	31	13	21	34	65
Ratio (cross 8)					0.954			1.046			2.000
9	P ^s P ^A P ^s P ^A	8	8	16	23	20	43	16	10	26	85
Ratio (cross 9)				0.753	2.024			1.223			4.000

(Fig. 8). These experiments show that *spectabilis* is a simple dominant to *succinea*.

The heterozygote of *spectabilis* and *axyridis* also resembles *spectabilis*, but can be distinguished from the homozygous *spectabilis* in that the anterior spot is smaller than that of the latter and often provided with accessory specks, one on each of its antero-lateral, postero-lateral and postero-median corners (Figs. 10, 11). From the mating, a homozygous *spectabilis* with a homozygous *axyridis*, are given only individuals like Figs. 10, 11 (Table IV, no. 4). But if a heterozygous *spectabilis* is used, about half of the F_1 individuals are *axyridis* (no. 8). The heterozygotes

inter se throw *spectabilis*, heterozygotes and *axyridis* in an approximately 1:2:1 ratio (no. 9). These findings show clearly that the genes for *spectabilis*, *axyridis* and *succinea* are in a multiple allelomorph series. P^s will be used for *spectabilis*.

Conspicua

Conspicua behaves very much like *spectabilis* when mated to *succinea* or to *axyridis*. The heterozygote of *conspicua* and *succinea* has a spot similar to the anterior spot of the heterozygote of *spectabilis* and *succinea* (Figs. 12, 13). The heterozygote of *conspicua* and *axyridis* also has a spot much resembling the anterior spot of the heterozygote of *spectabilis* and *axyridis* (Figs. 14, 15).

TABLE V

Nos. 10, 12, *conspicua* × *succinea*; no. 11, *conspicua* × *conspicua*

Cross no.	Parents	Progeny						
		<i>conspicua</i>			<i>succinea</i>			Total
		♀	♂	Total	♀	♂	Total	
10	P^cP^c pp	40	21	61	—	—	—	—
11	P^cP^c P^cP^c	49	31	80	15	11	26	106
Ratio (cross 11)				3.019			0.981	4.000
12	P^cP^c pp	3	1	4	2	1	3	7
Ratio (cross 12)				1.143			0.857	2.000

The cross, homozygous *conspicua* × *succinea*, gives only individuals like Figs. 12, 13 (Table V, no. 10); in F_2 *succinea* appears forming about one-quarter of the whole population (no. 11). The backcross gives the two types in a nearly 1:1 ratio (no. 12). It is thus clear that *conspicua* is a simple dominant to *succinea* like other types.

Heterozygotes of *conspicua* and *axyridis* mated together throw *conspicua*, heterozygotes and *axyridis* in an approximately 1:2:1 ratio (Table VI, no. 13). *Conspicua* mated with the heterozygote gave in an experiment 2 *conspicua*:1 heterozygote of *conspicua* and *axyridis*:1 *axyridis* (no. 14). Another experiment, where a heterozygote of *conspicua* and *axyridis* was crossed with *succinea*, produced *conspicua* and *axyridis* in about the same number (no. 15).

A homozygous *conspicua* mated to a homozygous *spectabilis* gives *conspicua* alone in F_1 (Table VII, no. 16), and *conspicua* and *spectabilis* in a 3:1 ratio in F_2 (no. 17). F_1 backcrossed gives *conspicua* and *spectabilis* in a 1:1 ratio (no. 18).

It is evident then that *conspicua* is another gene belonging to the same allelomorphic series as all the genes already mentioned, and largely dominant to any of these. P^c will be used for this gene.

TABLE VI

No. 13, heterozygote of *conspicua* and *axyridis* × heterozygote of *conspicua* and *axyridis*; no. 14, *conspicua* × heterozygote of *conspicua* and *axyridis*; no. 15, heterozygote of *conspicua* and *axyridis* × *succinea*

Cross no.	Parents	Progeny									Total	
		Heterozygote of <i>conspicua</i> and <i>axyridis</i>										
		<i>conspicua</i>			<i>axyridis</i>			<i>axyridis</i>				
		♀	♂	Total	♀	♂	Total	♀	♂	Total		
13	P^cP^A	P^cP^A	1	2	3	5	3	8	2	1	3	14
Ratio (cross 13)					0.857	2.286			0.857			4.000
14	P^cp	P^cP^A	11	11	22	5	5	10	2	8	10	42
Ratio (cross 14)					2.096	0.952			0.952			4.000
15	P^cP^A	pp	1	7	8	—	—	—	9	3	12	20
Ratio (cross 15)					0.800				1.200			2.000

TABLE VII

Nos. 16, 18, *conspicua* × *spectabilis*; no. 17, *conspicua* × *conspicua*

Cross no.	Parents	Progeny						
		<i>conspicua</i>			<i>spectabilis</i>			Total
		♀	♂	Total	♀	♂	Total	
16	P^cP^c P^sP^s	52	47	99	—	—	—	—
17	P^cP^s P^cP^s	83	91	174	27	27	54	228
Ratio (cross 17)				3.053	0.947			4.000
18	P^cP^s P^sP^s	57	55	112	48	54	102	214
Ratio (cross 18)				1.047	0.953			2.000

*Forficula*¹

This is one of the rarer pattern types occasionally seen among the specimens collected in the field. The pattern resembles that of *axyridis*, but differs from it in that the anterior five spots are confluent in the manner shown in Figs. 16, 17 and the posterior-most spot is smaller than in *axyridis*. The heterozygote of *forficula* and *succinea* often can be distinguished from the homozygotes in that the anterior spot has a black speck in it (Fig. 17). However, the speck is missing in some cases and the heterozygotes may show the same appearance as the homozygotes (Fig. 16). An individual of this type gave in a cross with *succinea*, *forficula*

¹ Name given by Prof. T. Komai.

alone (Table VIII, no. 19), which produced in the next generation *forficula* and *succinea* approximately in a 3 : 1 ratio (no. 20). The back-cross gave these types nearly in the same frequency (no. 21).

A heterozygote of *axyridis* and *succinea* mated to a heterozygote of *forficula* and *succinea* threw four different types, *axyridis*, heterozygote of *axyridis* and *forficula*, *forficula* and *succinea*, in nearly equal frequency (Table IX, no. 22). The backcross of the heterozygote with *succinea* gave

TABLE VIII

Nos. 19, 21, *forficula* × *succinea*; no. 20, *forficula* × *forficula*

Cross no.	Parents	Progeny						
		<i>forficula</i>			<i>succinea</i>			Total
		♀	♂	Total	♀	♂	Total	
19	P ^F P ^F pp	9	15	24	—	—	—	—
20	P ^F p P ^F p	17	5	22	3	7	10	32
Ratio (cross 20)		2.750			1.250			4.000
21	P ^F p pp	26	25	51	34	29	63	114
Ratio (cross 21)		0.895			1.105			2.000

TABLE IX

No. 22, *axyridis* × *forficula*; no. 23, heterozygote of *axyridis* and *forficula* × *succinea*

Cross no.	Parents	Progeny											
		<i>axyridis</i>			Heterozygote of <i>axyridis</i> and <i>forficula</i>			<i>forficula</i>			<i>succinea</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
22	P ^A p P ^F p	6	7	13	9	7	16	8	4	12	10	3	13
Ratio (cross 22)		1.963			1.185			0.889			0.963		
23	P ^A P ^F pp	11	6	17	—	—	—	10	5	15	—	—	—
Ratio (cross 23)		1.062						0.938					

axyridis and *forficula* in nearly the same number (no. 23). The heterozygote of *axyridis* and *forficula* resembles *axyridis*, but can be distinguished from it in that the intero-median spot is smaller than the other spots (Fig. 18). Likewise the heterozygote of *forficula* and *spectabilis* or *conspicua* can be told by the peculiar shape of the anterior spot which has an indentation at the antero-lateral corner (Figs. 19, 20).

A crossing experiment between *spectabilis* and *forficula* gave *spectabilis*, heterozygote of *spectabilis* and *forficula*, *forficula* and *succinea* in nearly

equal frequency (Table X, no. 24). Another experiment yielded from the same combination, heterozygote of *spectabilis* and *forficula* and *forficula* in 1:1 ratio (no. 25). The heterozygotes together threw *spectabilis*, heterozygote of *spectabilis* and *forficula* and *forficula* in an approximately 1:2:1 ratio (no. 26).

TABLE X

Nos. 24, 25, *spectabilis* × *forficula*; no. 26, heterozygote of *spectabilis* and *forficula* × heterozygote of *spectabilis* and *forficula*

Cross no.	Parents	Progeny												Total
		Heterozygote of <i>spectabilis</i> and <i>forficula</i>												
		<i>spectabilis</i>			<i>forficula</i>			<i>forficula</i>			<i>succinea</i>			
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	
24	P ^s p P ^F p	2	3	5	1	2	3	2	1	3	1	3	4	15
Ratio (cross 24)		1.333			0.800			0.800			1.067			4.000
25	P ^s p P ^F P ^F	—	—	—	5	8	13	5	7	12	—	—	—	25
Ratio (cross 25)					1.040			0.960						2.000
26	P ^s P ^F P ^s P ^F	3	11	14	11	22	33	8	8	16	—	—	—	63
Ratio (cross 26)		0.889			2.095			1.016						4.000

TABLE XI

No. 27, *conspicua* × *fortificula*; no. 28, heterozygote of *conspicua* and *fortificula* × *succinea*

Cross no.	Parents	Progeny												Total	
		Heterozygote of <i>conspicua</i> and <i>forficula</i>													
		<i>conspicua</i>			<i>forficula</i>			<i>forficula</i>			<i>succinea</i>				
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total		
27	P ^c p	P ^F p	7	5	12	13	3	16	11	7	18	7	6	13	59
Ratio (cross 27)			0.814			1.085			1.220			0.881			4.000
28	P ^c P ^F	pp	12	16	28	—	—	—	14	10	24	—	—	—	52
Ratio (cross 28)			1.077						0.923						2.000

The F_1 of a cross between *conspicua* and *forficula* consisted of 1 *conspicua*:1 heterozygote of *conspicua* and *forficula*:1 *forficula*:1 *succinea* (Table XI, no. 27). This shows that the parents had both been heterozygotes of *succinea*. A heterozygote of *conspicua* and *forficula* backcrossed to *succinea* produced *conspicua* and *forficula* in about the same number (no. 28).

All these experiments show clearly that *forficula* belongs to the same allelomorph series as that of all the other genes. **P^F** may be used as the symbol for it.

Transversifascia

Transversifascia is another rare type with a pattern as in Figs. 21, 22. Among the individuals of *transversifascia*, the heterozygote of *transversifascia* and *succinea* can be distinguished from the homozygote in that the spot has one or three black specks in it (Fig. 22). However, these specks are often missing, when the heterozygote cannot be distinguished from the homozygote (Fig. 21). The heterozygote of *transversifascia* and *axyridis* and that of *transversifascia* and *forcicula* are shown in Figs. 23 and 24 respectively.

In an experiment *transversifascia* \times *transversifascia*, *succinea* was segregated, forming about one-quarter of the population (Table XII, no. 29). *Transversifascia* \times *succinea* produced both types in 1 : 1 ratio (no. 30).

TABLE XII

No. 29, *transversifascia* \times *transversifascia*; no. 30,
transversifascia \times *succinea*

Cross no.	Parents	Progeny						
		<i>transversifascia</i>			<i>succinea</i>			Total
		♀	♂	Total	♀	♂	Total	
29	P ^T p P ^T p	13	15	28	4	3	7	35
Ratio (cross 29)				3:200			0:800	4:000
30	P ^T p pp	14	13	27	13	14	27	54
Ratio (cross 30)				1:000			1:000	2:000

It is thus evident that *transversifascia* is also a simple dominant to *succinea*.

The cross *transversifascia* \times *axyridis* produced in an experiment *transversifascia*, heterozygote of *transversifascia* and *axyridis*, *axyridis* and *succinea* in an approximately 1 : 1 : 1 : 1 ratio (Table XIII, no. 31), and in another, heterozygote and *axyridis* in a nearly 1 : 1 ratio (no. 32). The heterozygote backcrossed to *succinea* gave *transversifascia* and *axyridis* in 1 : 1 ratio (no. 33).

Transversifascia mated with *spectabilis* gave *conspicua*, *transversifascia*, *spectabilis* and *succinea* in an approximately 1 : 1 : 1 : 1 ratio (Table XIV, no. 34). One of the F_1 *conspicua* was mated to *succinea*, and threw only *transversifascia* and *spectabilis* in about the same numbers (no. 35). This result shows that the heterozygote of *transversifascia* and *spectabilis* cannot be distinguished from *conspicua* in its phenotype.

From a cross *conspicua* \times *transversifascia*, *conspicua*, *transversifascia* and *succinea* were produced in an approximately 2 : 1 : 1 ratio (Table XV, no. 36). This suggests that the heterozygote of *conspicua* and *trans-*

versifascia is phenotypically identical with *conspicua*. This suggestion is confirmed by another experiment in which one of the *conspicua* from no. 36 mated to *succinea* threw *conspicua* and *transversifascia* in a nearly 1 : 1 ratio (no. 37).

TABLE XIII

Nos. 31, 32, *transversifascia* × *axyridis*; no. 33, heterozygote of *transversifascia* and *axyridis* × *succinea*

Cross no.	Parents	Progeny											
		<i>transversifascia</i>			Heterozygote of <i>transversifascia</i> and <i>axyridis</i>			<i>axyridis</i>			<i>succinea</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
31	P^Tp P^Ap	1	4	5	1	5	6	3	1	4	4	—	4
Ratio (cross 31)				1.053	1.263			0.842			0.842		
32	P^Tp P^Ap^A	—	—	—	5	7	12	4	10	14	—	—	—
Ratio (cross 32)				0.923				1.077			2.000		
33	P^Tp^A pp	6	—	6	—	—	—	4	2	6	—	—	—
Ratio (cross 33)				1.000	1.000				1.000			2.000	

TABLE XIV

No. 34, *transversifascia* × *spectabilis*; no. 35, *conspicua* × *succinea*

Cross no.	Parents	Progeny											
		<i>conspicua</i>			<i>transversifascia</i>			<i>spectabilis</i>			<i>succinea</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
34	P^Tp P^Sp	13	7	20	13	12	25	8	11	19	9	12	21
Ratio (cross 34)				0.941	1.177			0.895			0.987		
35	P^Tp^S pp	—	—	—	1	4	5	2	5	7	—	—	—
Ratio (cross 35)				0.833				1.167			2.000		

TABLE XV

No. 36, *conspicua* × *transversifascia*; no. 37, *conspicua* × *succinea*

Cross no.	Parents	Progeny								
		<i>conspicua</i>			<i>transversifascia</i>			<i>succinea</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total
36	P^Cp P^Tp	4	5	9	3	2	5	2	2	4
Ratio (cross 36)				2.000	1.111			0.889		
37	P^Cp^T pp	3	13	16	8	5	13	—	—	—
Ratio (cross 37)				1.103	0.897			2.000		

A cross between *transversifascia* and *forficula* gave *transversifascia*, heterozygote of *transversifascia* and *forficula*, *forficula* and *succinea* in an approximately 1 : 1 : 1 : 1 ratio (Table XVI, no. 38). The heterozygote

mated to *succinea* segregated *transversifascia* and *forficula* in about a 1 : 1 ratio (no. 39).

These experiments show clearly that *transversifascia* is another allelomorph of all the genes stated. P^T may be used as the symbol for the gene.

This is another rare pattern type shown in Figs. 25, 26. The heterozygote of *aulica* and *succinea* often can be distinguished from the homozygote in that the spot encloses black specks (Fig. 26). But these specks are missing in some cases, when the heterozygote cannot be distinguished from the homozygote (Fig. 25). The heterozygote of *axyridis* and *aulica* resembles *axyridis*, but can be distinguished from it by the anterior two spots being very small or entirely absent (Fig. 27). The heterozygote of

TABLE XVI

No. 38, *transversifascia* × *forficula*; no. 39, heterozygote of *transversifascia* and *forficula* × *succinea*

Cross no.	Parents	Progeny												Total
		<i>transversifascia</i>			Heterozygote of <i>transversifascia</i> and <i>forficula</i>			<i>forficula</i>			<i>succinea</i>			
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	
38	P^Tp P^Tp	6	2	8	6	1	7	4	3	7	5	2	7	29
Ratio (cross 38)		1.102			0.966			0.966			0.966			4.000
39	P^TP^F pp	12	8	20	—	—	—	16	10	26	—	—	—	46
Ratio (cross 39)		0.870						1.130						2.000

Aulica

forficula and *aulica* and that of *transversifascia* and *aulica* are shown in Figs. 28 and 29 respectively. A heterozygote of *conspicua* and *axyridis* mated to a heterozygote of *aulica* and *succinea* threw three different types, *conspicua*, *axyridis* and heterozygote of *axyridis* and *aulica*, in an approximately 2 : 1 : 1 ratio (Table XVII, no. 40). The heterozygote of *axyridis* and *aulica* mated with *succinea* gave *axyridis* and *aulica* in 1 : 1 ratio (no. 41).

A cross of *spectabilis* with the heterozygote of *axyridis* and *aulica* gave *spectabilis*, heterozygote of *spectabilis* and *axyridis*, *axyridis* and *aulica* in a nearly 1 : 1 : 1 : 1 ratio, showing that the original *spectabilis* had been a heterozygote of *spectabilis* and *aulica* (Table XVIII, no. 42).

A mating between *forficula* and *aulica* gave *forficula*, the heterozygote of *forficula* and *aulica*, *aulica* and *succinea* in nearly equal frequency (Table XIX, no. 43).

The cross *transversifascia* × *aulica* produced *transversifascia*, *aulica* and *succinea* in an approximately 2 : 1 : 1 ratio (Table XX, no. 44). The heterozygote of *transversifascia* and *aulica* crossed with *succinea* produced *transversifascia* and *aulica* in an approximately 1 : 1 ratio (no. 45).

TABLE XVII

No. 40, heterozygote of *conspicua* and *axyridis* × *aulica*;
no. 41, heterozygote of *axyridis* and *aulica* × *succinea*

Cross no.	Parents	Progeny											
		<i>conspicua</i>			<i>axyridis</i>			Heterozygote of <i>axyridis</i> and <i>aulica</i>			<i>aulica</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
40	P ^{CP} A P ^{Au} P	6	8	14	7	2	9	5	3	8	—	—	—
Ratio (cross 40)				1.802	1.161			1.032			4.000		
41	P ^{AP} A ^u pp	—	—	—	6	8	14	—	—	—	9	5	14
Ratio (cross 41)					1.000						1.000		
											2.000		

TABLE XVIII

Spectabilis × heterozygote of *axyridis* and *aulica*

Cross no.	Parents	Progeny											
		<i>spectabilis</i>			Heterozygote of <i>spectabilis</i> and <i>axyridis</i>			<i>axyridis</i>			<i>aulica</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
42	P ^S P P ^{AP} A ^u	5	3	8	4	3	7	2	6	8	5	2	7
Ratio (cross 42)				1.067	0.933			1.067			0.933		
											4.000		

TABLE XIX

Forficula × *aulica*

Cross no.	Parents	Progeny											
		<i>forficula</i>			Heterozygote of <i>forficula</i> and <i>aulica</i>			<i>aulica</i>			<i>succinea</i>		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
43	P ^F P P ^{Au} P	5	5	10	5	6	11	6	2	8	6	4	10
Ratio (cross 43)				0.865	1.189			0.865			1.081		
											4.000		

Lastly, the cross, heterozygote of *transversifascia* and *aulica* × *aulica* produced *transversifascia* and *aulica* in an approximately 1 : 1 ratio (no. 46).

All these experiments show clearly that *aulica* belongs to the same allelomorphic series as that of all the other genes. P^{Au} will be used as the symbol for it.

TABLE XX

Nos. 44, 45, transversifascia \times *aulica*; no. 45,
transversifascia \times *aulica*

Cross no.	Parents		Progeny									Total
			<i>transversifascia</i>			<i>aulica</i>			<i>succinea</i>			
			♀	♂	Total	♀	♂	Total	♀	♂	Total	
44	P^Tp	P^{Au}p	18	20	38	6	14	20	11	8	19	77
Ratio (cross 44)			1.974			1.039			0.987			4.000
45	P^TP^{Au}	pp	3	5	8	7	5	12	—	—	—	20
Ratio (cross 45)			0.800			1.200						2.000
46	P^TP^{Au}	P^{Au}p	7	16	23	8	13	21	—	—	—	44
Ratio (cross 46)			1.045			0.955						2.000

SUMMARY AND DISCUSSION

All the experiments dealt with above show beyond any doubt that *succinea*, *axyridis*, *spectabilis*, *conspicua*, *forficula*, *transversifascia* and *aulica* are due to autosomal genes belonging to the same allelomorphic series. Of these, *succinea* is largely recessive to any other gene, but the heterozygotes between it and the dominant types can often be distinguished from the respective homozygotes by the peculiarity in the shape of the anterior spot or because the spot encloses some specks, though there are cases where the heterozygote cannot be distinguished from the homozygote. Heterozygotes between *axyridis* and the other dominant types can also be told from the homozygotes, though *axyridis* is largely recessive to *spectabilis* or *conspicua*. *Conspicua* is completely dominant to *spectabilis*. Of the rarer types, *forficula*, *transversifascia* and *aulica*, *forficula* is largely recessive either to *spectabilis* or to *conspicua*, but the heterozygote can be distinguished from the homozygote by a peculiarity in the anterior spot.

Transversifascia is completely recessive to *conspicua*, and the heterozygote between it and *spectabilis* cannot be distinguished from *conspicua* phenotypically.

Aulica is completely recessive either to *spectabilis* or *conspicua*, and it is largely recessive to *transversifascia*, though the heterozygote can be distinguished from the homozygous *transversifascia*.

The fact that all the seven genes belong to the same allelomorphic series, instead of being independent of one another, is especially clear in

the experiments like nos. 9, 13, 17 or 26, where both parents are heterozygous for two dominant genes—whereas on the independent gene hypothesis the double recessive type *succinea* should have appeared among the progeny.

Tan & Li's data can also be mostly accounted for by the multiple-allelic hypothesis, except for three crosses (nos. 5, 56, 57) where a few *succinea* were obtained, besides *conspicua* and *spectabilis*, from the inbreeding of the heterozygous *conspicua*.

All these, however, are presumably due to experimental errors. Such errors could easily happen, especially in the early stages of the larva, unless great care is taken not to mix different broods. It may be added also that in the well-known experiments of *Phytodecta variabilis* by Zulueta (1925), the different pattern types were shown to be due to allelomorphic genes which are sex-linked instead of being autosomal.

I wish to express my hearty thanks to Prof. T. Komai and Dr M. Chino for their kindness in many ways. The present investigation was carried out partly with an aid from the Society for the Promotion of Scientific Research of Japan. It is my pleasure to express my gratitude to the Society.

REFERENCES

- DOBZHANSKY, TH. (1924). "Die geographische und individuelle Variabilität von *Harmonia axyridia* Pall. in ihren Wechselbeziehungen." *Biol. Zbl.* **44**, 401-21.
- (1933). "Geographical variation in lady-beetles." *Amer. Nat.* **67**, 97-126.
- HOSINO, Y. (1933). "Variation in the elytral color patterns of the lady-bird beetles." *Zool. Mag., Tokyo*, **45**, 255-67.
- (1936). "Genetical studies of the lady-bird beetle, *Harmonia axyridis* Pallas (Report II)." *Jap. J. Genet.* **12**, 307-20.
- (1939). "Genetical studies of the lady-bird beetle, *Harmonia axyridis* Pallas (Report III)." *Jap. J. Genet.* **15**, 128-138.
- TAN, C. C. & LI, J. C. (1932-3). "Variation in the color patterns in the lady-bird beetles, *Ptychanatis axyridis* Pallas." *Peking nat. Hist. Bull.* **7**, 175-93.
- (1934). "Inheritance of the elytral color patterns of the lady-bird beetle, *Harmonia axyridis* Pallas." *Amer. Nat.* **68**, 252-65.
- ZULUETA, A. DE (1925). "La herencia ligada al sexo en el coleoptero *Phytodecta variabilis* (Ol.)." *Eos*, **1**. (Cited by Morgan, T. H. (1928) in *The Theory of the Gene*.)

UMBROUS: A CASE OF DOMINANCE MODIFICATION IN MICE

By K. MATHER

John Innes Horticultural Institution, Merton

AND S. B. NORTH

Galton Laboratory, University College, London

(With Two Text-figures)

I. THE SOURCE OF UMBROUS

IN 1934 an extensive linkage backcross in mice was initiated at the Galton Laboratory (Fisher & Mather, 1936). Among other characters the agouti-non-agouti difference was segregating in this experiment, but in addition to the usual agouti and non-agouti mice certain intermediate individuals were observed. The first mouse of the intermediate type to be recognized as anomalous was a male which occurred in a litter together with a normal black and a normal black-agouti. At about two weeks old the anomalous mouse was recognizably intermediate between its two sibs. Though it was clearly not a pure black it was definitely darker in general appearance than was the agouti sib. At maturity this mouse could be seen to be an agouti, but along its back extending from the eyes to the tail ran a dark streak in which the hairs had the subterminal yellow ticking, characteristic of agouti, absent or much reduced.* On the sides and ventral surface the mouse showed the normal agouti appearance. The dark streak of the back was not sharply delimited from the normally pigmented flanks.

Several apparently similar cases of aberrant agoutis had previously been reported, notably that of Barrows (1934), which he described as a case of dominance modification. None of these cases were, however, investigated sufficiently for the details of the inheritance and interactions of the agouti modifier to be known. Hence it was decided to make a detailed analysis of this new case. It was decided to call the character "umbrous" following Barrows's terminology, but his usage of "dark" for the umbrous phenotype as opposed to the gene or genes concerned was considered confusing and was not employed.

Before proceeding to a consideration of the breeding behaviour and progeny of this umbrous mouse it is desirable to give some account of its

230 *Umbrous: a Case of Dominance Modification in Mice*

ancestry. A stock of mice had been kept by Prof. R. A. Fisher for a number of years. Selection and inbreeding had been exercised in order to separate lines differing in their constitution for modifiers of the piebald phenotype. Agouti and non-agouti had been kept segregating in the stock for several years. There was no record of umbrous mice having appeared. In 1934 two wavy brown non-agouti males were obtained from Dr Grüneberg. These were from the original wavy line of Prof. F. A. E. Crew. The umbrous male described above was directly descended from crosses of these males with the piebald stock. It seems reasonably certain that the umbrous character was introduced by one or both of these males, the failure to notice it earlier in the experiment being due to their progeny containing mainly dilute and brown mice, on which background umbrous is not so clearly visible. After the finding of the first umbrous male several other mice were observed to show the character. These all agreed in being descended from the brown wavy males. Furthermore, an intensive search, carried out over several years, in the piebald stock has failed to bring to light any umbrous individuals. So there can be little doubt that the umbrous did not and does not exist in this stock.

2. THE BREEDING BEHAVIOUR OF UMBROUS

The umbrous male first found in 1934 was mated to two black non-agouti females from the piebald stock. The two crosses gave 19 full-coloured and 9 dilute mice. Umbrous could not be classified with certainty on the nine dilutes, but the other 19 mice (black and brown) were divisible into: 2 normal agouti, 8 umbrous agouti, 9 non-agouti. Two of the eight umbrous agouti mice were mated back to their father and gave, in addition to 7 dilutes:

6 agouti, 7 umbrous agouti, 9 non-agouti.

Thus a mating of umbrous \times umbrous can give non-umbrous, indicating that the umbrous allelomorph is dominant in the sense of the *Drosophila* usage. There might be more than one umbrous gene, but only one will be assumed for the present. The interpretation of the first cross is that the original umbrous male (known from its ancestry to be heterozygous agouti Aa) was heterozygous for dominant umbrous (Uu) and the two females to which it was mated were $aa uu$. This would give one-quarter $Aa uu$ (agouti), one-quarter $Aa Uu$ (umbrous agouti), one-quarter $aa uu$ (non-agouti) and one-quarter $aa Uu$, which would presumably appear to be ordinary non-agouti. The figures obtained agree reasonably well, though 2 : 8 is but an indifferent fit to the expected 1 : 1.

The second crosses were, on this interpretation, **AaUu** × **AaUu**, but before the observed segregations could be fully understood it was necessary to know more details of the interaction of **A** and **U**, particularly the phenotype of **AAUu** mice. Hence mice from these and similar later crosses were tested by mating to normal non-agouti (i.e. **aa****uu**) stock mice if they were themselves agouti, or to ordinary agouti (**Aa****uu**) stock mice if they were non-agouti. Table II shows the segregation observed among seventeen progeny from **AaUu** × **AaUu** crosses tested in this way.

TABLE I

	AA	Aa	aa	Total
UU	1	1	1	3
Uu	3	5	1	9
uu	3	1	1	5
Total	7	7	3	17

The non-agouti mice, though phenotypically uniform, were referable to three classes on crossing to agouti stock animals, according to whether their agouti progeny (all **Aa** of course) were all normal, all umbrous or half of each kind. The agouti animals, some of which were normal and some umbrous, were separable into six classes, according to whether they were **AA** or **Aa**, and to their umbrous constitution. The occurrence of these nine classes leaves no doubt that umbrous is a modifier of agouti, unable to show on non-agouti. The original classification of the various agoutis into umbrous and not umbrous was, however, not completely consistent. **AaUu** and **AaUU** mice were found always to have been called umbrous, but the **AAUu** mice were sometimes supposed to be non-umbrous and sometimes called a doubtful umbrous. Inasmuch, however, as the genotype tests employed only involved phenotypic separation of **Aa****uu** and **AaUu** mice, a distinction which could always be made with relative ease, the six genotypic classes of agouti were separated with confidence by this means.

The segregation of 3 pure-breeding umbrous, 9 heterozygous umbrous and 5 not umbrous mice strongly supports the assumption that a single gene is responsible for the umbrous condition. Further evidence is supplied by the segregations observed in the progeny of the heterozygous umbrous animals tested by crossing to stock mice. These heterozygous umbrous mice were of three kinds, **AA**, **Aa** and **aa**. The former gave 24 normal agouti and 29 umbrous agouti progeny in crossing to **aa****uu** mice. From the **AaUu** × **aa****uu** crosses 23 normal agouti, 11 umbrous agouti and 28 non-agouti progeny were obtained, and from the **aaUu** mice, crossed

232 *Umbrous: a Case of Dominance Modification in Mice*

to **Aauu** testers, 15 agoutis were raised of which 8 were normal agouti and 7 umbrous agouti.

The agouti progeny may be set out as in Table II and χ^2 calculated for an expectation of 1 : 1.

TABLE II

	Au	AU	χ^2	D.F.
From AAUu \times aaau	24	29	0.4717	1
AaUu \times aaau	23	11	4.2353	1
aaUu \times Aauu	8	7	0.0667	1
Total	55	47	0.6275	1

The χ^2 analysis is then:

	χ^2	D.F.	P
Deviation	0.6275	1	0.50-0.30
Heterogeneity	4.1462	2	0.20-0.10
Total	4.7737	3	

The χ^2 for the **AaUu** \times **aaau** cross is large when taken by itself, but ceases to have any significance when analysed in conjunction with the other data.

These results leave little doubt that the umbrous distinction is determined by a single gene in our stocks.

Other data for various crosses involving agouti and umbrous are given in Table III. In many cases the segregations are not informative, as the classification into umbrous and not umbrous is not easy in the **AA** mice, and mistakes have most probably been made. The only reliable data are those in which umbrous is classified on **Aa** mice as in the test matings described above. The matings of **AAUU** \times **AAUU** and **AaUU** \times **AAUU** are of some interest as showing that the 48 **AAUU** and **AaUU** mice in the progenies were always classifiable as umbrous.

There is no evidence of linkage between **A** and **U**, such as Barrows suspected. Matings of the type **aaau** \times **AaUu** have given 25 normal agouti (**Au**), 19 umbrous agouti (**AU**), 37 non-agouti (**a**). This does not differ significantly from the 1 : 1 : 2 expected on the basis of no linkage. Further the deviation is in the direction opposite to that expected from the known constitution of the double heterozygotes which were $\frac{\text{AU}}{\text{au}}$.

3. THE INTERACTION OF AGOUTI AND UMBROUS

It was clear from the early crosses, described above, that the interaction of agouti and umbrous is somewhat complex. In order to make a more detailed analysis it was necessary to be able to produce mice known from their ancestry to be of given genotypes for **A**, **a** and **U**, **u**. Lines of

the four homozygous types **AAUu**, **AAuu**, **aaUU** and **aauu** were first obtained from tested mice and these were then intercrossed in the various ways necessary to produce all the nine genotypic combinations of **A**, **a** and **U**, **u**. A number of mice of each genotype were skinned at the age of 14 days and a further set at 6 weeks. The comparisons discussed below are based entirely on these skins, from which the umbrous effects are more accurately recordable than from living animals.

TABLE III

Each line is concerned with the type of mating shown on the left, and may include data from a number of matings. All dilute progeny are omitted as they are unclassifiable for umbrous

Type of mating	Agouti		Non-agouti	Total
	Normal	Umbrous		
aa u u × Aa U u	2	8	9	19
aa U u × Aa U u	3	5	9	17
aa U U × Aa U U	—	—	5	5
aa U U × AA U U	—	12	—	12
Aa U u × Aa U u	17	29	10	56
Aa U U × Aa U U	—	144	51	195
Aa U U × AA U u	—	7	—	7
Aa U U × AA U U	—	5	—	5
AA U u × AA U u	8	5	—	13
AA U U × AA U U	—	43	—	43

The two sets of skins, 14 days and 6 weeks, agree in having the same interaction of agouti and umbrous. The younger ones are in general rather darker, presumably because the agouti yellow markings have not fully developed, but the differences between the various genotypes are shown equally by the skins of both ages.

All the **aa** skins are black, irrespective of their constitution in respect of umbrous. The situation in the agouti mice was more complex. First it became clear, by comparison of a number of both genotypes, that, contrary to the usual statement, agouti is not completely dominant, on the non-umbrous (**uu**) background. **Aa**u skins show a slight darkening down the medial line, as compared with **AA**u. The difference is certainly impossible to see with any regularity on living mice, but when a number of skins of each type are compared the distinction is clear, though some overlapping occurs. When the umbrous gene is present in the heterozygous state, heterozygous agouti (**Aa**U **u**) is markedly darker than in non-umbrous (**Aa**u) animals. The homozygous agouti (**AA**U **u**) is, however, hardly affected. A careful comparison shows it to resemble the **Aa**u phenotype rather than the **AA**u. The difference between **AA**u and **AA**U **u** is, then, not easily visible, especially on living mice. The

234 *Umbrous: a Case of Dominance Modification in Mice*

heterozygous umbrous shows an almost perfect case of dominance modification. It hardly affects the homozygous agouti, but renders the heterozygote definitely intermediate.

Turning to homozygous umbrous, both homozygous and heterozygous agouti are affected. **AAUU** is visibly darker than either **AAUu** or **AAuu**, and **AaUU** is darker even than **AaUu**. The two types **AAUU** and **AaUU** are, however, separable, **AaUU** being markedly darker than **AAUU**. This conclusion was verified by keeping a line of **AaUU** \times **AaUU** breeding for five generations. In each generation the progeny were classified into the three groups, not agouti (**aaUU**), very umbrous agouti (**AaUU**) and umbrous agouti (**AAUU**). The parents of the next generation were chosen from the very umbrous class, i.e. the one presumed to be **AaUU**. Their agouti constitution was then checked by the presence or absence of non-agouti (**aa**) in their progeny. From 13 matings chosen in this way not one failed to produce **aa** offspring. One male was used with three different females, hence the 13 matings involved 24 mice. All of these proved to be heterozygous agouti as expected from their phenotype. [We may unconsciously have selected the darkest very umbrous type, i.e. those most likely to be **AaUU**, for breeding tests.] In addition to these 13 matings, five others of the same type were set up, but mice known from their previous history to be **AaUU** were used (the 13 crosses were descended from these). All together the 18 matings gave 224 offspring, of which 29 were dilute and hence not used, umbrous being difficult to classify on dilute agouti. The remaining 195 consisted of: 51 not agouti (**aa**), 88 very umbrous agouti (**Aa**), 56 umbrous agouti (**AA**). $\chi^2 = 2.1077$ for deviation from a 1 : 2 : 1, which for two degrees of freedom is clearly not significant. This χ^2 may be analysed into two parts, each having one degree of freedom. The first part testing the agouti-non-agouti segregation, where a 3 : 1 ratio is expected, is 0.1385 and not significant. The second part testing the supposed **Aa-AA** segregation (expectation 2 : 1) is found to be 1.9692, and is not significant. Thus all the evidence is in agreement with the supposition that in **UU** mice the **aa**, **Aa** and **AA** classes of mice are distinguishable, **A**, **a** being incompletely dominant.

In order to appreciate the details of the interaction of agouti and umbrous it is necessary to form some idea of the relative lightness of the nine genotypic combinations of these two genes. This is not easy, and the values given below must be taken as arbitrary inasmuch as they are not derived from measurement but are merely grades as judged by eye. A number of our colleagues have given their opinions on the relative

lightness shown by the skins. Though these have not always agreed completely with our own views, shown in Table IV, below, they have been in sufficient agreement to give us some confidence in putting forward relative numerical values. There is, of course, no doubt as to the order in which the genotypes should be put on the grounds of relative lightness of the skin.

The numerical values which we believe to represent the darkening effect are:

TABLE IV

Relative lightness of the hair

	AA	Aa	aa
UU	10	8	0
Uu	11	10	0
uu	12	11	0

0 = black. 12 = wild type agouti.

The chief points of doubt are (a) whether **AaUU** should be shown as 8 or 9 and (b) whether 0-8 is a fair estimate of the difference between black and the darkest umbrous-agouti. As to the former point, we have been more influenced by the differences in the region of the head and shoulders than were those of our colleagues who suggested 9 as a value. This is a reflexion of our experience with living mice, on which the head region is the most easily utilizable area for record purposes. The difference between black and the darkest umbrous-agouti is most difficult to assess, but in any case is large as compared with the differences between agouti types.

This may be represented in a diagram (Fig. 1). The diagram is in effect a modified dosage-effect graph. The dosage of agouti is shown along the abscissa, and the phenotypic effect along the ordinate. The curve relating dose and effect may be supposed to remain constant in shape for all the umbrous genotypes. The action of umbrous then becomes what is effectively a change of scale along the abscissa. Thus in **uu** one dose of **A** (i.e. **Aauu**) is shown at 4 along the abscissa, double dose (**AAauu**) at 8, but in **Uu** the corresponding points are 2 and 4 and in **UU** 1 and 2. It is as if **Uu** had half the scale of **uu**, and **UU** half the scale of **Uu**. The curve relating dosage and effect is not completed near the origin, as its lower end is entirely unexplored. There may be a threshold below which no agouti effect can be produced even though some genotypic agouti potentialities are present. The important feature of this diagram is that it shows how a similar change applied to **Aa** and **AA** can alter the dominance relations of this gene pair. With such modifications of a gene's action as that por-

236 *Umbrous: a Case of Dominance Modification in Mice*

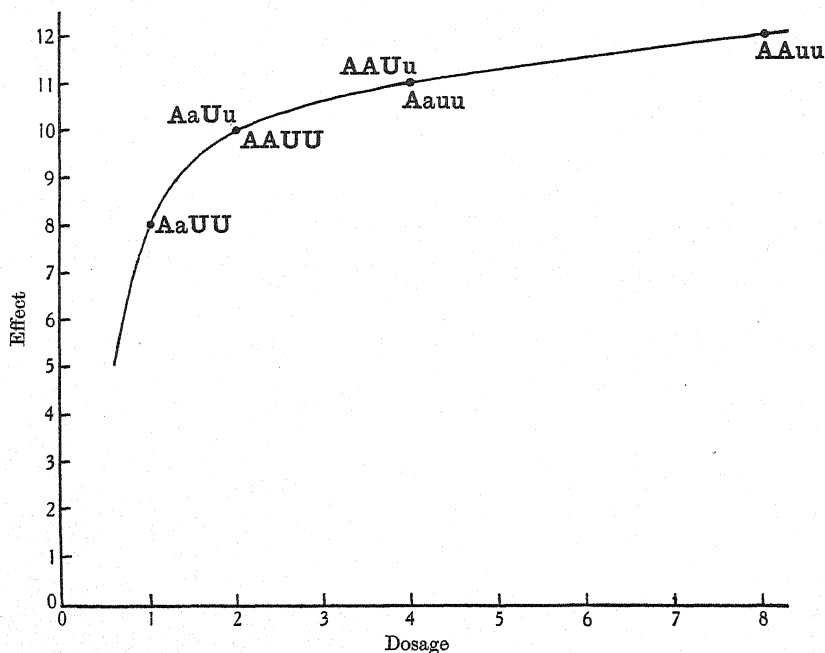


Fig. 1. Curve relating phenotype to agouti "dosage". The homozygous type **AA** is shown, in all cases, as having twice the dosage of the corresponding heterozygote **Aa**. Thus **AAUu** is at dosage 4 while **AaUu** is at 2. Heterozygous umbrous is portrayed as halving the dosage as compared with that at non-umbrous, while homozygous umbrous halves the dosage as compared with the heterozygote. Thus **AAuu** is at dosage 8, **AAUu** at 4 and **AAUU** at 2. For further description see text.

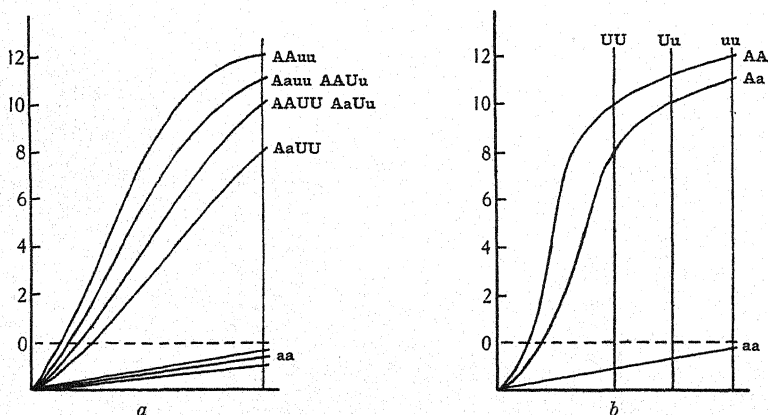


Fig. 2. Diagrams illustrating the possible types of interactions between **A**, **a** and **U**, **u**. (a) Here umbrous is shown as reducing the speed of the agouti reaction. (b) Here umbrous is shown as reducing the time over which the agouti reaction proceeds. In both cases the phenotypic effect is marked along the ordinate and time along the abscissa. The dotted horizontal line marks the threshold below which no agouti effect is produced. The upright lines mark the time of closure of the reaction.

trayed for umbrous, any effect on the heterozygote **Aa** must be accompanied by some corresponding effect on the homozygote **AA**. The change in dominance is not due to alteration of the effect of **Aa** while **AA** and **aa** remain constant. Such modification would require an alteration in the shape of the curve; it could not be achieved by simply altering the scale with respect to the representation of dosage as was done above.

Though the above diagram represents the interaction of agouti and umbrous in an orderly way, it does not lead directly to any physiological interpretation. We might suppose, with Goldschmidt, that the dosage effect of **A** is one of quantity leading to reactions of proportional rates. Then the "agouti" determining reaction would proceed twice as fast in **AA** as in **Aa** (though, of course, the reaction may be infinitely faster than that in **aa**) since **AA** has twice the dose of **Aa**. On this interpretation umbrous might have either, or both, of two effects, viz. (a) to affect the rate of the agouti reaction, but to do it proportionately in all genotypes, i.e. so that **Uu** mice would have the reaction proceeding at half the speed found in **uu** mice, though **AA** would still have a reaction of twice the speed of **Aa**; or (b) to affect the time over which the reaction progressed. With the closure applied at an earlier point in **Uu** as compared with **uu** mice, **AA** might still be twice as fast as **Aa** and yet the final effect would be less in both cases. To separate these two variables is impossible with the present data, but it is clearly the prime necessity for a fuller understanding of umbrous. It may also be noted that the simple assumption of the **A** dosage determining the rate of the agouti reaction is difficult to justify and may require modification. Fig. 2 shows the types of curve which would follow from the above interpretations of the action of **A** and **U**. It is virtually impossible to obtain a fit to the basic numerical data and get reasonable curves with slopes in a 2 : 1 : 0 ratio. This would appear to indicate that the simple assumptions made about the action of **A** are not true. It would, however, require less arbitrary and more extensive numerical data before any detailed analysis could be undertaken. The present results closely parallel those of Goldschmidt (1938) on dominance modification in the vestigial series of *Drosophila melanogaster*, where the data are more adequate for this type of analysis.

In the foregoing discussion it has been assumed that the agouti and umbrous genes are primary and secondary respectively in the sense that **A** determines the occurrence of the reaction while **U** may only modify the reaction if occurring. It is clear that there is a difference of "order" between the action of the two genes, as they are not transposable in any diagram of their action, i.e. even if it is possible to re-draw a given dia-

238 *Umbrous: a Case of Dominance Modification in Mice*

gram with **U** in place of **A** and **A** in place of **U**, the curves obtained are quite different. The assignment of **A** to the primary role is a simple reflection of the fact that the **U**, **u** difference has no expression in **aa** mice.

4. THE MODIFICATION OF DOMINANCE

Various hypotheses have been advanced in order to explain the phenomenon of dominance. Wright (1934) has argued that dominance is an inevitable consequence of the chemical reactions involved in the expression of the genes. More interesting, however, are the various selection hypotheses, the first of which was advanced by Fisher (1928), later variants being those of Haldane (1930) and Muller (1932). Fisher's hypothesis comprised two points: (a) that the degree of dominance of a gene is a character subject to selective influence, and (b) that the effect of selection would in the great majority of cases result in the accumulation of modifiers tending to make the heterozygote resemble the more advantageous homozygote, the "wild type" form. Both Haldane and Muller accepted Fisher's first principle but have proposed alternatives to his second. Haldane suggests that the chief effect of selection will be to increase the frequency of the more potent wild type allelomorphs, as such hyper-wild type allelomorphs will give heterozygotes more nearly resembling the homozygote.

Muller's hypothesis differs more from those of Haldane and Fisher than do these from one another. Muller supposes that the phenomenon of dominance is incidental, the main effect of selection being to lower the variability of the homozygote by making it more extreme in expression. The heterozygote then also becomes more extreme in expression in parallel with the homozygote, and dominance results. Either allelomorphs or modifiers may be selected for their action in this way.

Now reference to Fig. 1 shows that the dominance of a gene, measured by the ratio of the **Aa**-**aa** difference to the **AA**-**aa** difference, can be altered in two ways. If it be taken for granted that the dosage-effect curve of a gene is of a given shape, then dominance may be changed by moving the "dosage" of the gene along to the right, whereupon the difference in expression between homo- and heterozygote diminishes. This is the type of change produced by the umbrous gene-pair. It is characteristic that the expressions of both homo- and heterozygote are affected.

The second way in which dominance may be modified is by an alteration of the shape of the "dosage-effect" curve. To demonstrate the occurrence of this type of modification it would be necessary to show that

the heterozygote could be affected while both homozygotes remained of constant expression. Even in such cases it might be argued that the change was not really one of shape, one homozygote having altered, though only potentially; its expression remaining constant owing to a threshold effect.

Clear cases of dominance modification of the first kind are known, for example the present case and the vestigial dominigenes (Goldschmidt, 1938). It might be expected that Haldane's hyper-wild type allelomorphs would act in this way, too, but the known cases of such allelomorphs, vestigial (Goldschmidt, 1938) and white eye (Muller, 1935), though clearly cases of dominance modification, are inconclusive as to the nature of the change.

No clear example of the second type of dominance change, by alteration in the shape of the curve, has been described. Such cases may, perhaps, be rarer than those of the first kind, because they would involve a more complex physiological rearrangement. Where the modification is to be represented as due to the points moving along a dosage curve of fixed shape, the change may be supposed to involve a simple alteration in the speed of the reaction or reactions concerned. Where the dosage-effect curve is completely changed it would seem to be necessary to postulate a change in the whole balance of metabolic processes. It is difficult to see how this could be brought about by variation in the strength of allelomorphs of the type Haldane envisages; though Fisher's modifiers presumably could act in this way. In any case Fisher's and Haldane's hypotheses are in no sense mutually exclusive.

As stated above, Muller's view stands in rather marked contrast to the other two. Granted the initial premise, that extremity of expression is accompanied by reduced chance variability, any of the above cases could be interpreted in accordance with his hypothesis. It is to be presumed that if dominance modification is dependent on selection acting in this way, it will generally involve no change in the shape of the dosage-effect curve (cf. Muller, 1935).

The real difficulty in accepting Muller's account of the indirect action of selection on degree of dominance lies in the initial assumptions of the selective advantage of extreme expression and the existence of a correlation between extreme expression and low variability. It seems doubtful whether extreme types always enjoy a selective advantage over other less extreme kinds. In the case of polygenic characters, as for example stature in man, it seems certain that the intermediate types must have been the most successful ones in each population. With regard to the

second assumption, if one supposes that there is a threshold expression above which increase in dosage can produce no increase in actual effect, the reduction in phenotypic variability is understandable. In such a case increase of effective dosage increases the potential expression, though not increasing actual expression, and chance variation in potential is less likely to cause a drop below the threshold and so result in a real phenotypic change. Though such thresholds are doubtless common it is difficult to accept their existence in all cases of dominance. In *Pisum sativum* tall is dominant to short and yet there is no justification for believing that a tall threshold exists, since it is known that tall types are subject to both genetical and environmental variability. In such a case apparently no threshold exists for the character. Furthermore, if tall is completely dominant to short, as it appears to be, we have a case of complete dominance of a gene whose effect is highly variable. This seems difficult to interpret on Muller's hypothesis. It appears that much more evidence linking variability and expression is necessary before any confidence can be placed in the supposed indirect selection of dominance through its relation with the variability of the homozygote. There can, however, be no doubt that dominance is subject to selective control, as Fisher postulated, and direct selection dependent on the difference in expression between the various genotypes will explain all the facts available at present.

SUMMARY

The gene umbrous is a modifier of the expression of agouti. It has no visible effect in non-agouti animals, but makes agouti mice darker. Heterozygous agouti is affected more than homozygous agouti. Heterozygous umbrous has a definite effect, but it is not so marked as that of homozygous umbrous. In homozygous umbrous mice, homozygous and heterozygous agouti animals are separable by eye, whereas in non-umbrous mice they are not. Hence umbrous may be regarded as a dominance modifier of agouti.

Umbrous appears to act by controlling the rate or degree of progress of the reaction or reactions whose occurrence is determined by the agouti gene.

Dominance is a character subject to selective control, acting directly through the difference in phenotype of the heterozygote and homozygote, rather than indirectly through a correlation of degree of dominance and variability of the homozygote.

REFERENCES

- BARROWS, E. F. (1934). "Modification of the dominance of agouti to non-agouti in the mouse." *J. Genet.* **29**, 9-16.
- FISHER, R. A. (1928). "The possible modification of the response of the wild type to recurrent mutation." *Amer. Nat.* **62**, 115-26.
- (1935). "Dominance in poultry." *Philos. Trans. B*, **225**, 195-226.
- (1938). "Dominance in poultry. Feathered feet, rose comb, internal pigment and pile." *Proc. roy. Soc. B*, **125**, 25-48.
- FISHER, R. A. & MATHER, K. (1936). "A linkage test with mice." *Ann. Eugen.*, *Lond.*, **7**, 265-80.
- GOLDSCHMIDT, R. (1938). *Physiological Genetics*. New York: McGraw Hill.
- HALDANE, J. B. S. (1930). "A note on Fisher's theory of the origin of dominance, and on a correlation between dominance and linkage." *Amer. Nat.* **64**, 87-90.
- MULLER, H. J. (1932). "Further studies on the nature and causes of gene mutation." *Proc. 6th internat. Cong. Genet.* **1**, 213-55.
- (1935). "On the incomplete dominance of the normal allelomorphs of white in *Drosophila*." *J. Genet.* **30**, 407-14.
- WRIGHT, S. (1934). "Physiological and evolutionary theories of dominance." *Amer. Nat.* **68**, 24-53.

Note added in proof

Since the foregoing was written, Prof. Punnett has drawn our attention to certain members of the **E** series of allelomorphs, which determine the occurrence of what he terms "refractory black" in rabbits (see Punnett (1930), *J. Genet.* **23**, 265-74). These genes are indistinguishable in the absence of agouti, all the animals being black. But combined with agouti, heterozygous or homozygous, various degrees of suppression of the agouti character are found. It might appear that the "umbrous" phenotype was similarly due to the production of refractory black in the mice, but the resemblance between the mouse and rabbit cases is most probably of a superficial nature, for three reasons:

1. The umbrous effect is most marked on the head and shoulders, whereas this is one of the last areas to show agouti suppression in rabbits.

2. The rabbit genes have a much more extreme effect on agouti than does umbrous in the mouse.

3. Umbrous is a dominance modifier of agouti, but in the rabbit heterozygous and homozygous agouti are apparently acted on equally, though the refractory black genes themselves show incomplete dominance.

K. M.
S. B. N.



VARIATION AND GENETICS OF THE AWN IN *TRITICUM*

BY A. E. WATKINS AND SYDNEY ELLERTON

School of Agriculture, Cambridge

(With Plates X-XII and Two Text-figures)

CONTENTS

	PAGE
I. Introduction	243
II. Preliminary analysis of factors affecting awn length . . .	244
III. Awn variation in the different species	246
IV. Earlier investigations on awn inheritance	252
V. New genetic data:	
(1) Introduction and list of parents and crosses . . .	255
(2) Dominance relations	257
(3) Awn inheritance in <i>T. vulgare</i> and <i>T. sphaerococcum</i> . .	258
(4) The hooded character of Abyssinian tetraploids . . .	264
VI. Discussion	265
Summary	267
References	268
Explanation of Plates X-XII	270

I. INTRODUCTION

MANY genetic studies have been made of beardedness and beardlessness in wheat, but their mode of inheritance is still far from being fully understood. This is chiefly because none of the work so far done has been based on a careful study of the awn types to be found throughout the range of the wheat species. Most workers have determined the mode of inheritance of the characters in some isolated cross or crosses which happen to have been made for plant-breeding purposes. The description of the parental types used has usually been inadequate, the contrasting phenotypes being loosely termed "bearded" and "beardless". In some of the more recent work and in one or two early papers, true beardless forms have been distinguished from those with very short awns (awn tips), and "half-awned" types have also been recognized. Even here, however, no distinction has been made between certain pairs of genetical types which are easily distinguishable phenotypically; and it is difficult to tell, except in cases where good illustrations or exceptionally good descriptions of the wheats used are given, precisely which types have been used as parents.

There are several reasons why the bearded character has received so much attention; it is an obvious and striking character, with simple inheritance in the first crosses studied, though it later proved to be complicated; it is important agriculturally and therefore to the breeder; it is the first of a hierarchy of characters used by systematists for classifying the hexaploid wheat species into "botanical varieties"; and, lastly, it proved to be connected with the speltoid mutations which have been so extensively studied by geneticists and cytologists.

With regard to the classification of wheats into botanical varieties, four awn classes were recognized by Vavilov (1923): *aristatum* (awned), *muticum* (awnless), *breviaristatum* (half-awned) and *inflatum* (hooded). Later Hosono (1934) suggested that the *inflatum* class should be divided into two, *aristoinflatum* and *mutinflatum*, which will be called hooded bearded and hooded beardless for the purposes of the present paper. It will be shown by the present work that these five classes, while distinguishing some of the major awn types which may be recognized by the systematist, are not in each case a single genetical type but may be groups of unrelated but superficially similar types, some of which may be distinguished accurately by visual inspection alone.

As pointed out above, the literature on wheat-awn genetics is confused in that the exact types used are rarely accurately described. It therefore seems advisable to give first a careful description of the major awn types occurring in the genus. In a majority of cases, it may then be determined from descriptions and photographs here given which genetic types are involved, and the earlier literature may be summarized on an exact basis.

II. PRELIMINARY ANALYSIS OF FACTORS AFFECTING AWN LENGTH

Before describing the various awn types that form the basis of the present genetical study, it must be pointed out that the character is subject to several distinct types of genetical influence. These may be classified under four headings as follows:

- (i) Chromosome number.
- (ii) Modifying genes.
- (iii) Genes that have their most striking effect in influencing some other character, but also have a marked effect on awn length.
- (iv) The major awn genes proper.

The effect of chromosome number is very striking. The tetraploid wheat species in general have very much longer awns than the hexaploids. Very long awns are found in *Triticum dicoccum* and *T. turgidum*,

and *T. durum* contains types with longer awns than any other wheat. Percival (1921) gives the awn length of the two tetraploid species *T. durum* and *T. turgidum* as 11–23 cm. and 8–16 cm., while the corresponding figures for *T. vulgare* and *T. Spelta*, two hexaploid species, are given as 5–10 and 6–8 cm. respectively.

In the segregates from pentaploid hybrids this effect of chromosome number is equally striking. The tetraploid segregates of every awn type have longer awns or awn points than the corresponding hexaploid segregates. The exact mechanism of this effect is not known.

Even within one species and within the types that would be classified without hesitation as “fully bearded”, there are considerable variations in awn length. These are presumably due to modifying genes. The magnitude of their effect may be judged from the following figures quoted from Percival (1921) for the awn length of different forms of various species, all of which would be classified as fully bearded. A few widely different lines have been selected to give an idea of the extent of the range. Awn lengths are in centimetres.

TABLE I
Awn lengths (cm.) in fully bearded wheats
(data from Percival (1921))

Tetraploid		Hexaploid	
<i>T. dicoccum</i>	<i>T. durum</i>	<i>T. turgidum</i>	<i>T. vulgare</i>
6–8. <i>farrum</i> (White Emmer)	12. <i>murciense</i> (form 3)	8–9. <i>dinurum</i> (Red Rivet)	4–6. <i>ferrugineum</i> (Carman)
7–9. <i>farrum</i> (Large White Emmer)	11–13. <i>apulicum</i> (“Cawnpore 18”)	9–10. <i>dinurum</i> (Nonette de Lausanne)	5–6. <i>meridionale</i> (form 1)
12–15. <i>liguliforme</i>	14. <i>murciense</i> (Santa Marta)	12–13. <i>dinurum</i> (Trigo focense)	7–8. <i>ferrugineum</i> (Hsu Hsu Mai)
15–17. <i>majus</i>	15–16. <i>melanopus</i> (Trigo Andaluze)	12–14. <i>iodurum</i> (Petianelle noire de Nice)	8–9. <i>erythroleucon</i> (Perle de Nuise-ment)
	17–18. <i>erythro-melan</i> (Medea)		

Pl. X, A and B, show the extreme forms of fully bearded *vulgare*, and a form with average awn length is shown in D.

Corresponding differences in the length of the awn tips are found in tip-awned and half-bearded wheats, and occasionally make the exact classification of a particular form difficult.

It has been shown conclusively by Stewart and his co-workers (Stewart, 1926, 1928 a; Stewart & Bischoff, 1931; Stewart & Heywood, 1929) that in all the cases tested genes that modify ear density also modify the awn length of fully awned wheats. Correlation between rachis

internode length and awn length was highly significant, e.g. $+0.6500 \pm 0.028$ in the cross Kanred \times Sevier, both fully awned forms. It may be noted that the *compactum* gene reduces awn length appreciably, in the same way as the other spike density genes tested. This correlation doubtless accounts for some of the differences in awn length between different forms of the same species, shown in the above table, though there are undoubtedly other modifying genes also.

None of these modifying genes has been studied in the present work.

Thirdly, we have a number of genes or very closely linked gene groups that have their most marked effect on some plant character other than awns, but which at the same time influence awn development to a marked degree. Among the most striking of these genes (or, more probably, closely linked gene groups) are those distinguishing *T. polonicum* from *T. durum* and *T. sphaerococcum* from *T. vulgare*. Both these have a very marked effect in reducing awn length.

T. polonicum, according to Percival (1921), has an awn length of 7–12 cm., as compared with the 11–23 cm. given for the nearly related *T. durum*. In crosses with other species the long glume *polonicum* gene **P** is seen to cause a marked shortening of the awns; e.g. Matsumura (1936) in the cross-bearded *T. polonicum* \times tip-awned *T. Spelta* shows that both tip-awned and bearded *polonicum* segregates have shorter awns than the corresponding *Spelta* types.

T. sphaerococcum shows an even more marked reduction in the length of the awn, the genetically fully bearded types having awns only 1.5–2 cm. long, as compared with a length of 5–10 cm. in *T. vulgare*. This gene group has an effect on awn length as great as that of some of the major genes that influence awn length alone. A typical fully bearded *T. sphaerococcum* is shown in Pl. X C, in comparison with fully bearded forms of *T. vulgare*.

The fourth class, the genes that have their major effect in modifying the length of awn, are the ones with which the main part of the present genetical work is concerned. The phenotypes to which they give rise are described in the next section.

III. AWN VARIATION IN THE DIFFERENT SPECIES

The diploid wheats exhibit no major variation with respect to awn type; all forms are fully bearded, and the group therefore has little interest in the present connexion and may be left out of consideration.

The tetraploid species are also uniformly fully bearded, except for the shortening of the awns in *T. polonicum* already referred to and for a very

distinct group of wheats confined to certain regions of Abyssinia. These were first studied in detail by Vavilov *et al.* (1931) and referred by them to special subsections of *T. durum* and *T. turgidum*. Among them several distinct awn types are found. Fully bearded forms exist as everywhere else, but "hooded", half-awned and tip-awned types also occur.

A hooded Abyssinian wheat (*T. turgidum abyssinicum* Vav.) is illustrated in Pl. XII. The character is somewhat complex in its effects. The length of the awns in the main tillers is considerably less than in bearded forms, and the awns are often bent at the base in a characteristic way. The glumes are inflated. In the late tillers the awns are still further reduced to a hook, or are bent round on themselves to form a close spiral; they are very much thickened, and in some forms have prominent lateral membranous outgrowths. All these wheats have considerably shorter straw than more typical *T. durum* and *T. turgidum* from the Mediterranean region. The difference between the early and late tillers of one and the same plant in these hooded forms is very striking. In general, the tillers developed latest show the strongest effect, and the character is more strongly exhibited in material sown late in spring than in autumn-sown material.

The half-awned Abyssinian tetraploid wheats do not show this difference between the early and late tillers. All the ears have long awns at and near the apex, but the awn length is progressively reduced as one approaches the base of the ear, where there are awn tips only a few millimetres long.

The tip-awned forms described by Vavilov *et al.* (1931) have awnlets 1 cm. or so long at the apex of the ear and very short awn tips towards the base. The type is very like the ordinary "beardless" *vulgare* wheats of western Europe in this respect.

In Vavilov's paper a number of wheats are figured which are hooded but have much shorter awns than the hooded type figured in Pl. XII. They present much the same appearance as the late tillers of that type. If the ears figured are actually main tillers, then we have another category "hooded beardless", which would be expected to occur where the genes for the hooded and for the half-awned or tip-awned condition occur together. None of these forms actually exists in the collection of some 200 Abyssinian wheats at Cambridge, though all the other types are represented. Their existence would, however, be expected. In the following discussion of the genetics of the various awn types, the ordinary hooded type will be called "hooded bearded" in contradistinction to "hooded beardless". "Hooded bearded" wheats are those that contain

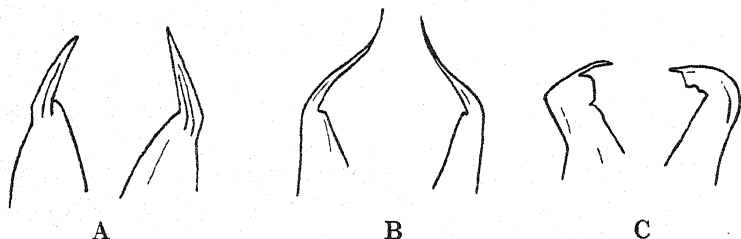
248 *Variation and Genetics of the Awn in Triticum*

the gene for hooded and no other major genes that reduce awn length; they differ from fully bearded only by a single gene pair.

The hexaploid wheats show even greater awn variation than the tetraploids. The full range is shown in *T. vulgare*, which will be discussed first. In it we can distinguish completely beardless wheats, two distinct forms with short awn tips, half-bearded types and forms that may be described as "hooded bearded" and "hooded beardless", as well as the usual fully awned wheats. This makes seven types in all, which are described separately below and illustrated in Pls. X and XI.

(1) *Tipped 1*

The two different types of tip-awned wheats may be designated as tipped 1 and tipped 2. Tipped 1 is the type of "beardless" wheat familiar in England, and studied by Biffen (1905) and other early workers.



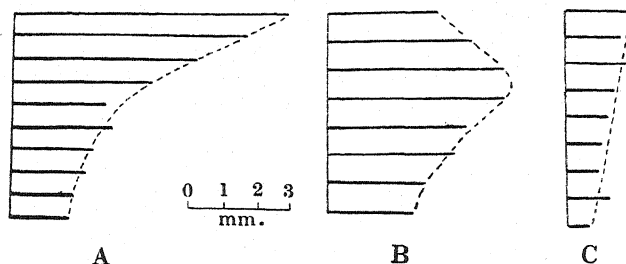
Text-fig. 1. Characteristic forms of awn tips in *T. vulgare*. A. Tipped 1 (var. Swedish Iron). B. Tipped 2 (var. Mesopotamian *vulgare*). C. Hooded beardless (var. Chinese White).

Wheats of this type have very short awn tips on the paleae at the base of the ear and in the centre, but towards the apex they become increasingly long, and in the apical quarter of the ear may be 1 cm. long or even longer, though in most types the longest tips do not reach this length. These awn tips are usually quite straight and not bent at the base, but sometimes there is a sharp angle at the junction of awn and palea or just above it. Even in these cases, however, the distal part of the awn is straight or nearly so, and not hook-like. The distribution of the awn tips with the longer awns confined to the apex of the ear is characteristic of this type. The type of bent awn tip sometimes found in this category is shown in Text-fig. 1 A.

(2) *Tipped 2*

The other type of tip-awned wheat, tipped 2, is quite different. Here the awn tips are much more nearly equal in length all along the ear. If there is any difference, the longest tips are to be found near the centre of the ear and not at the apex. The awn tip is often curved, the most com-

mon form of curvature being shown in Text-fig. 1 B. It is never curved right round on itself as in "hooded beardless" wheats, and it never has membranous lateral outgrowths. Text-fig. 2 shows a comparison between the distribution of awn tips in typical ears of the tipped 1 and tipped 2 categories and also of beardless *T. vulgare*. Measurements were made of the length of the longest awnlet in each spikelet on one side only of three typical ears of each class, and the average values represented in the diagram.



Text-fig. 2. Length and distribution of awn tips in tipped 1, tipped 2 and beardless *T. vulgare*. For description see text.

(3) *Beardless*

Completely beardless wheats are comparatively infrequent. Here the awn tips are reduced almost to extinction. They are very little if any longer than the short blunt teeth found on the empty glumes, at most 1 or 2 mm.

Pl. XI A, B and C show typical wheats of the tipped 1, tipped 2 and beardless categories.

(4) *Half-awned*

The half-awned *vulgare* wheats vary considerably among themselves, and the character may perhaps be determined by at least two different genes. In most cases (cf. Pl. XI D) the awns in the apical portion of the ear are relatively long, but they become progressively shorter towards the base, where they may be reduced to quite short awn points. Some forms, however, have short awns of practically equal length (usually an inch or so) from the apex and the base of the ear, while others show extremely long awns in the apical four or five spikelets with very short ones below. No exact classification of these types was possible on the basis of visual examination, since they showed all gradations from one to another. All of them differ sharply from the next class by the fact that

there is no marked difference in awn expression between the main and subsidiary tillers of the same plant.

(5) *Hooded bearded*

The hooded bearded type in *T. vulgare* is that classed by Hosono (1934) as *aristoinflatum*. It corresponds quite closely to the condition already described in Abyssinian tetraploid wheats. The awns of the main tillers are reduced in length in much the same way as in the half-awned wheats. They are, however, curved and deformed, usually with a marked inflexion at the base; often they resemble a shepherd's crook in shape. Frequently, though not always, the awn is considerably broadened at the base and has membranous lateral expansions, giving an appearance rather like that of the hooded barleys (Pl. XI E-H).

The glumes have a peculiar shape which Vavilov (1923) calls "inflated", but which is difficult to describe adequately. The glume is more markedly convex than in most wheats, and is rather tough, so that the ear is often difficult to thresh. The most striking and apparent difference is that the spikelets seem to have a U-shaped outline, the midribs of the two empty glumes lying almost parallel to each other for the distal two-thirds of their length, instead of diverging to give a more V-shaped outline (cf. Pl. XI E, F). This distinction in outline is not, however, apparent in most Chinese hooded wheats, owing to the large number of grains per spikelet present (Pl. XI G, H).

Sometimes, in spite of the above differences, it is a little difficult to distinguish between the main tillers of hooded and half-awned types, at least until the eye has become accustomed to the rather subtle difference in the shape of the glumes. The infallible test is then to look at the late tillers. Here the half-awned types show no marked difference from the main tillers, while in the hooded plants the expression of hoodedness is considerably exaggerated: the awns are reduced in length to a strongly recurved awn tip which usually has very marked membranous lateral outgrowths (Pl. XI H).

(6) *Bearded*

The fully awned wheats need no special description; they are easily distinguished from the foregoing two types by the fact that the awns towards the base of the ear are long, as well as those at the apex (Pl. X A, B, D).

(7) *Hooded beardless*

There is still one other category to be described, that of "hooded beardless". This resembles hooded bearded except that the awn length is still further reduced, the awn being represented by a short hook, usually with membranous lateral outgrowths. The variety Chinese White (Text-fig. 1 C and Pl. XI K) is typical of this class.

Thus we have in all seven different easily recognizable categories of awn development in *vulgare* wheats. It will be shown later that three of these types (tipped 1, tipped 2 and hooded bearded) are each due to a single gene difference from the recessive fully bearded. The half-awned wheats also have a single gene difference from bearded, but the same gene is not involved in every case—two genetically different types within the class have been established. The remaining two types, beardless and hooded beardless, are due to various combinations of two (or possibly in some cases three) of the genes responsible for the other types: in all the cases studied they show a two-gene difference from bearded.

The geographical distribution of the above seven types has been studied in some detail and will be described in a later communication. Although the difference between types are in general very clear-cut, an occasional difficulty in classification does arise. A very few wheats have the distribution of their awn tips intermediate between typical tipped 1 and typical tipped 2; and it is difficult to decide whether certain forms with very short awn tips should be described as tip-awned or beardless. These doubtful plants, however, form an extremely small proportion of the whole and are certainly not sufficiently frequent to vitiate studies of the geographical distribution of the types.

In *T. compactum* tip-awned, hooded, half-bearded and bearded forms occur, corresponding exactly to the similar forms in *T. vulgare*. All the forms, however, have rather shorter awns or awn tips than the corresponding *vulgare* type, owing to the effect of the *C* gene in reducing awn length. As far as we are aware, no completely beardless forms of *compactum* have been specifically reported, and the literature does not enable one to distinguish between tipped 1 and tipped 2—they are classed together as "*muticum*". It is probable, however, that a complete parallel series actually exists. The species shows its greatest diversity in Afghanistan, and half-awned and hooded forms were first discovered there by Vavilov & Bukinich (1929) (see also Kobelev, 1928). Owing to this parallelism between *vulgare* and *compactum*, and to the fact that a much larger and more varied amount of *vulgare* material was available

at Cambridge, genetic experiments were confined to the former species. It seems unlikely that *compactum* contains any major gene for awns not studied in *vulgare*.

T. Spelta is probably a recent form almost confined to mountainous regions of Central Europe (Flaksberger, 1930); it contains bearded forms and beardless forms with apical awnlets (tipped 1).

T. sphaerococcum contains forms with short awns (1.5–2 cm.) which are genetically fully bearded. It also contains tipped forms; in all these the awn tips are very short owing to the marked shortening effect of the *sphaerococcum* gene complex, and it is impossible to distinguish different phenotypes within them. In the forms tested genetically, the beardless type has proved to be tipped 2 (Ellerton, 1939). The Cambridge collection contains no hooded forms of *sphaerococcum*, and they have not been reported in the literature.

IV. EARLIER INVESTIGATIONS ON AWN INHERITANCE

As stated above, it is intended here to interpret earlier literature on the basis of the awn types described in the foregoing section. No reference is found in the literature to studies of the awn types found in Abyssinian tetraploid wheats, so that the following discussion refers only to those found in the hexaploid group.

The cross tipped 1 \times bearded has been studied by Biffen (1905), Clark & Quisenberry (1929), Gaines (1917), Gaines & Singleton (1926) and many others (cf. Matsuura, 1933). In each case the F_1 resembled the tip-awned parent more nearly than the bearded one, so that the tipped condition may be regarded as dominant. The F_1 plants of different crosses were not of exactly the same awn type, however. In some cases they had awn tips distinctly longer than those of the tipped 1 parent, and it was possible to classify the F_2 into a 1 : 2 : 1 ratio. In others, the heterozygotes could only be accurately distinguished from the pure tipped 1 plants by their breeding behaviour and the F_2 was classified in the ratio 3 tip-awned : 1 bearded. It appears that the various wheats used differ in certain genes which modify the dominance of the tipped 1 gene.

Kajanus (1923, 1927) reported crosses between tipped 1 and bearded in which there was a deficiency of bearded segregates in F_2 and F_3 . He attributed this to differential viability of the bearded plants, particularly with respect to their cold resistance. Watkins (1928) also found a deficiency of bearded segregates in a *T. Spelta* \times *T. vulgare* cross.

The gene determining the tipped 1 condition is known to be linked to the genes for keeled glumes, for squarehead ear type and for pubescent

nodes. Kajanus (1923) reported 33 % of crossing-over with the keeled glume gene. Watkins (1928) found the value to be 28 % in a *Spelta* × *vulgare* F_2 and 39 % in a *turgidum* × *vulgare* backcross. Nilsson-Leissner (1925) gave the cross-over value as 35 % and Nilsson-Ehle (1927) recorded a gametic ratio of 1 : 2.8 : 2.8 : 1, equivalent to 26 % of crossing-over. Philiptschenko (1934) found that the cross-over value between tipped 1 and the gene *S*, determining the keeled glume or speltoid condition, was 33 %, while there was 35 % of crossing-over between tipped 1 and the gene *q* for the squarehead ear type. The tipped 1 gene lay between the other two genes, *S* and *Q*.

For the linkage with pubescent node, Gaines & Carstens (1926) obtained a cross-over value of 5 %, while Love & Craig (1924) found almost complete linkage.

In the cross tipped 1 × beardless, Clark & Hooker (1926) found a 3 : 1 ratio in F_2 for the segregation of the major gene concerned, and also found evidence of the segregation of a modifying gene. Stewart & Tingey (1928) obtained transgressive segregation due to the presence of modifying genes in this combination, tipped 1 segregates being obtained which had longer awn tips than the tipped 1 parent Marquis.

The tipped 2 type has been studied in crosses to a much smaller extent. Love & Craig (1926) reported a 15 : 1 ratio for tip-awned and beardless to bearded in a cross between Sonora (tipped 2) and a tipped 1 wheat. A similar cross involving Sonora was made by Quisenberry & Clark (1933) who obtained an F_2 ratio of 104 awned : 1710 intermediate : 276 awnless. In the F_3 only half of the awnless group bred true, the others segregating tipped plants. The true ratio therefore corresponded closely to the 1 : 14 : 1 expected on the basis of independent segregation of two genes. The tipped 1 and tipped 2 genes in combination clearly produce a true beardless type.

The same authors crossed Sonora with a bearded wheat and obtained an abnormal single-gene ratio with a deficiency of tipped 2 segregates in the F_2 .

The genetics of completely beardless wheats has been studied by several authors. The above crosses with Sonora suggest that some such wheats may differ from bearded by two gene pairs, which separately determine the tipped 1 and tipped 2 conditions. The variety Supreme studied by Quisenberry & Clark (1933) appears to have been of this constitution, since in crosses with Sonora (tipped 2) a single-gene segregation for beardless *v.* tip-awned was obtained and no half-awned or other new types appeared.

The beardless wheats studied by other authors, however, all seem to have differed from bearded by two genes which individually determine a half-awned and the tipped 1 conditions. This is true of the beardless Indian wheats, Pusa 4 and A 88, used in crosses with bearded by Howard & Howard (1912, 1915) and the varieties Federation (Stewart & Heywood, 1929), Hard Federation (Stewart & Judd, 1931; Clark, 1924; Clark *et al.* 1928) and Bobs (Clark *et al.* 1928). In each of these cases the factors for tipped 1 and half-awned segregated independently.

A very unusual result was obtained by Stewart (1926, 1928 *b*) who in crosses between Federation and the bearded varieties Sevier and G-149 found that the two-awn genes of Federation were linked with 35 % of crossing-over. In another Federation cross (Stewart & Heywood, 1929) the two genes for beardless showed independent inheritance as in the other bearded \times beardless crosses described above. For a final explanation to be given, the lines used by Stewart would have to be tested with lines of known genetical constitution, but it may be suggested that the two genes were involved in a reciprocal translocation in Stewart's material, so that in one of his bearded lines they were carried in the same chromosome and in all other lines were in different chromosomes. In some ways an explanation of this kind is more probable than that suggested by Stewart, who gave Federation the formula **aa bb tt** and the two different bearded lines **AA BB tt** and **AA bb TT**, **A** and **T** being linked and independent from **B**. This interpretation demands that a cross between the two bearded lines would give a proportion of **AA tt bb** plants, which would be half awned on Stewart's hypothesis. But in all crosses reported in the literature, as well as in the large number that have come under the notice of the present writers, this has never been known to happen, the cross bearded \times bearded having always given nothing but bearded.

The half-awned condition discussed in all the foregoing crosses clearly depends on a single-gene difference from bearded, the factor not normally being linked with tipped 1. The cross half-awned \times tipped 1 (Pusa 7 \times Pusa 6) was studied by Howard & Howard (1915), and the expected 1 : 14 : 1 segregation of beardless : various intermediates : bearded was obtained.

Half-awned wheats of this type are apparently different from others which have been recorded from time to time as rare admixtures in the wheat varieties of north-western Europe. These have been reported by Jenkin (1925) in Hen Gymro and by Nilsson-Ehle (1920) in Extra Squarehead II, Panzerweizen and Sommerperlweizen. The latter half-

awned types were studied genetically by Nilsson-Ehle, who showed that they arose by mutation and bore a multiple allelomorph relation with the gene for tipped 1. The crosses half-awned \times bearded, half-awned \times tipped 1 and tipped 1 \times bearded all gave 3 : 1 ratios, the type with the shorter awns being dominant in each case. 42 % of crossing-over between half-bearded and keeled was found (Nilsson-Ehle, 1927), and this corresponds roughly with the average value of about 35 % found by various authors between tipped 1 and keeled glumes, giving another indication of the multiple allelomorph relation between the half-awned gene concerned and tipped 1.

There therefore appear to be two factors that determine half-beardedness, as is also the case with the tip-awned condition. In the latter case, the two types may be distinguished phenotypically with accuracy, in the former they cannot.

This survey covers all the major awn types described in the foregoing section except those of the Abyssinian tetraploid wheats and the hooded bearded and hooded beardless *vulgare* types. No reference to crosses involving these types occurs in the literature.

V. NEW GENETIC DATA

(1) *Introduction and list of parents and crosses*

The genetic behaviour of the hooded awn type found in Abyssinian tetraploid wheats and of all the awn types found in *T. vulgare*, with the exception of half-awned, was studied in the crosses to be described in this section. The omission of half-awned *vulgare* was unfortunate. It was due to the fact that in 1936, when most of the crosses were made, the authors had not properly appreciated the distinction between the half-awned and hooded bearded types; and several forms used as parents and thought to be half-awned at the time actually proved to be of the other type.

The following parental forms were used. Most of them were unnamed varieties taken from the wheat collection grown in Cambridge and are designated by the collection numbers:

A. *Hexaploid species*

Bearded:

1. Persia 170 (*T. vulgare* var. *ferrugineum* Al.).
2. *T. sphaerococcum* var. *rubiginosum* Perc.

Tipped 1:

3. Squarehead's Master (*T. vulgare* var. *millurum* Al.).
4. Swedish Iron (*T. vulgare* var. *lutescens* Al.).
5. Benefactor (*T. vulgare* var. *leucospermum* Körn.).

256 Variation and Genetics of the Awn in Triticum

Tipped 2:

6. M.V. (= Mesopotamian *vulgare*) (*T. vulgare* var. *leucospermum* Körn.).
7. Persia 465 (*T. vulgare* var. *pyrothrix* Al.).
8. India 742 (*T. sphaerococcum* var. *tumidum* Perc.).
9. India 740 (*T. sphaerococcum* var. *tumidum* Perc.).
10. India 39 (*T. vulgare* var. *lutescens* Al.).
11. China 29 (*T. vulgare* var. *lutescens* Al.).

Beardless:

12. Australia 6 (*T. vulgare* var. *albidum* Al.).

Hooded bearded:

13. Persia 357 (*T. vulgare* var. *rufinflatum* Flaksb.).
14. Persia 413 (*T. vulgare* var. *albinflatum* Flaksb.).
15. Persia 368 (*T. vulgare* var. *chorassanicum* Vav.).

Hooded beardless:

16. Chinese White (*T. vulgare* var. *huangyangense* Hosono.).

B. Tetraploid species

Bearded:

17. Indian Runner (*T. durum* var. *provinciale* Al.).
18. Cambridge Rivet (*T. turgidum* var. *iodurum* Körn.).
19. Emmer (*T. dicoccum* var. *farrum* Körn.).

Hooded bearded:

20. Hooded Abyssinian (*T. turgidum abyssinicum* Vav.).

The following is a list of the crosses between these forms which were studied (the female parent is placed first in each case):

A. Crosses involving awn genes of *T. vulgare* or *T. sphaerococcum*

Tipped 1 × bearded:

1. (Swedish Iron × Rivet) F_1 back-crossed to both parents.
2. (Swedish Iron × Emmer) F_1 back-crossed to Emmer and to Rivet.

Tipped 2 × bearded:

3. Persia 465 × Persia 170.
4. (M.V. × Rivet) F_1 back-crossed to both parents.

Tipped 1 × tipped 2:

5. M.V. × Squarehead's Master.
6. India 740 × Squarehead's Master.

Tipped 2 × tipped 2:

7. Persia 465 × M.V.
8. M.V. × India 742 and reciprocal.

Hooded bearded × bearded:

9. Persia 357 × Persia 170.

Hooded bearded × tipped 2:

10. India 39 × Persia 368.
11. Persia 413 × India 39.
12. China 29 × Persia 368.
13. Persia 357 × Persia 465.

Hooded beardless × bearded:

14. Chinese White × *T. sphaerococcum* var. *rubiginosum*.
15. (Chinese White × Rivet) F_1 backcrossed to both parents.

Hooded beardless \times tipped 2:

16. Chinese White \times M.V.

Hooded beardless \times tipped 1:

17. Chinese White \times Squarehead's Master.

Beardless \times bearded:

18. (Australia 6 \times Indian Runner) F_1 \times Indian Runner.

B. *Crosses involving the hooded Abyssinian tetraploid type*

Hooded bearded \times bearded:

19. Abyssinian Hooded \times Indian Runner.

Hooded bearded \times tipped 1:

20. (Benefactor \times Abyssinian Hooded) F_1 back-crossed to both parents.

(2) *Dominance relations*

Most authors have regarded the beardless or tip-awned condition in wheat as being dominant to bearded, though the opposite view has also been taken (Howard & Howard, 1912, 1915; Stewart, 1926, etc.). Actually, none of the awn genes is completely dominant or completely recessive. The F_1 plants of crosses between tipped 1 or tipped 2 and bearded have awn tips distinctly longer than the tip-awned parent, but much nearer to it than to the bearded parent. The same applies to crosses between beardless or hooded beardless and bearded forms. It is therefore clear that the bearded type should receive the recessive symbol in each of these cases. As indicated above, the exact degree of dominance of the beardless or tip-awned condition varies from cross to cross, although the F_1 is much nearer to the parental type with the shorter awns.

Crosses between the hooded bearded and bearded types show a curious state of affairs. The main tillers of heterozygous plants are practically indistinguishable from bearded, whereas the late tillers appear hooded. The hooded condition may therefore be almost dominant or almost recessive in different parts of one and the same plant. This applies to the hooded type found in Abyssinian tetraploid wheats as well as to that found in *T. vulgare*. For the sake of uniformity, the bearded type will be symbolized as recessive to hooded in the following treatment.

In crosses between tip-awned *vulgare* wheats and bearded *T. sphaerococcum*, the short beards (1.5–2 cm. long) of the latter appear in F_1 to be completely dominant. This, however, is because short-awned *T. sphaerococcum* is genetically fully bearded, the recessive *sphaerococcum* gene *s* itself reducing awn length; a bearded *sphaerococcum* plant roughly resembles a heterozygous bearded tip-awned *vulgare* plant in length of awn, since two *s* genes or a single tip-awn gene reduce awn length to about

258 *Variation and Genetics of the Awn in Triticum*

the same extent. In F_2 , in the *vulgare* and *sphaerococcum* segregates taken separately, the tip-awned condition is dominant as usual.

There seems little doubt that the awned condition should be regarded as the basic or primitive one in wheat, since it is found in all species and is the only one in diploid and tetraploid wheats with the exception of the Abyssinian group of tetraploids. The other types may have been derived from the bearded form by a series of incompletely dominant mutations, or one or more may have been present in one of the parent diploid forms from which the polyploid species may be presumed to have arisen.

(3) *Awn inheritance in T. vulgare and T. sphaerococcum*

(a) *Tipped 1 and bearded.*

It is clear from numerous results reported in the literature, some of which have been cited above, that tipped 1 and bearded differ by a single gene. Results for cross 1, *vulgare* \times *turgidum*, have already been given by Watkins & Cory (1931), very regular segregations being obtained when the F_1 was backcrossed to one or other of the parents. Larger numbers are now available, and are given below together with results for cross 2, *vulgare* \times *dicoccum*.

TABLE II
Segregation for tipped 1 and bearded

(1) Iron \times Rivet		
	Bearded	Tipped 1
$F_1 \text{ } \varnothing \times \text{Rivet } \sigma$	183	185
$F_1 \text{ } \varnothing \times \text{Iron } \sigma$	45	47
Iron $\varnothing \times F_1 \text{ } \sigma$	59	56
Rivet $\varnothing \times F_1 \text{ } \sigma$	46	44
Total	333	332
(2) Iron \times Emmer		
	Bearded	Tipped 1
$F_1 \text{ } \varnothing \times \text{Emmer } \sigma$	94	105
$F_1 \text{ } \varnothing \times \text{Rivet } \sigma$	21	16
Total	115	121

Tipped 1 will be denoted by B_1B_1 and bearded by b_1b_1 . In the above crosses B_1 and b_1 show significant linkage with the genes for keeled and rounded glumes, the cross-over percentage being about 41 (Watkins, 1928, 1940).

(b) *Tipped 2 \times bearded.*

This segregation was studied in crosses 3 and 4. In the former there was an unusually sharp distinction between the heterozygote and both

parental types in the F_2 , so that complete classification for awn type was possible. The segregation was:

	Bearded	Heterozygous	Tipped 2	Total
Observed	65	230	108	403
Expected	100.75	201.50	100.75	403
$\chi^2 = 17.24$. D.F. = 2. $P < 0.01$.				

A single-gene segregation is therefore indicated, but there is a significant deficiency of the bearded type. In this cross, only 73% of the F_2 grains sown produced mature plants, so that there may have been a selective elimination of bearded plants. A heavy late spring frost and a severe spring drought occurred during the season (1938) in which the family was grown.

Cross 4 gave the following backcross ratios for the segregation of tipped 2 *v.* bearded:

	Tipped 2	Bearded
(M.V. \times Rivet) $F_1 \times$ M.V.	17	24
(M.V. \times Rivet) $F_1 \times$ Rivet	19	25
Total	36	49

$$\chi^2 = 1.694. \quad P = 0.19 \text{ approx.}$$

In this case there was no evidence of linkage between the genes for tipped 2 *v.* bearded and round *v.* keeled glumes, there being 41 parental combinations, and 42 recombinations of these two gene pairs.

(c) *Tipped 1* \times *tipped 2*.

This combination was studied most extensively in cross 5, which was carried to F_3 . F_2 consisted of 315 beardless and tipped : 30 fully bearded, expectation on a 15 : 1 basis being 323.5 : 21.5, $\chi^2 = 2.92$ and P is approximately 0.1. There was some doubt about the classification of a few of the bearded plants with small ears or broken awns, but fifteen of these selected at random all bred true to fully bearded except that four families contained 1, 1, 2 and 2 tipped plants respectively; these exceptions were sharply set off from the remaining plants and were attributed to natural crossing.

Out of 109 beardless and tipped F_2 plants there were selected five thought to be quite beardless, i.e. to contain all four tipped genes, and six that were doubtful, the remaining ninety-eight all being judged to contain one or more tipped genes. The first five bred true to quite beardless as expected, and the remaining six doubtfuls gave five families that all contained tipped plants. Thus, out of 109 plants five were found to be true beardless and seven would be expected on a 15 : 1 basis.

These results show a two-gene difference between Mesopotamian

260 *Variation and Genetics of the Awn in Triticum*

vulgare and Squarehead's Master, the parents of cross 5. If the tipped 2 gene is denoted by B_2 we have

Tipped 1	$B_1B_1b_2b_2$
Tipped 2	$b_1b_1B_2B_2$
Beardless segregates	$B_1B_1B_2B_2$
Bearded segregates	$b_1b_1b_2b_2$

The same combination was studied in cross 6 between Squarehead's Master and India 740 and the F_2 segregation was:

	Bearded	Beardless and intermediate
Observed	46	806
Expected	53	799

χ^2 for this deviation is 0.86 and $P = 0.30 - 0.50$, so that here again we have a normal 15:1 ratio.

(d) *Tipped 2* \times *tipped 2*.

Crosses between the tipped 2 form M.V. used in cross 5 and the forms Persia 465 and India 742, the latter a tipped *T. sphaerococcum*, gave no segregation for awn type in the F_2 , indicating that both these wheats were also of the tipped 2 constitution, $b_1b_1B_2B_2$.

(e) *Hooded bearded* \times *bearded*.

The F_2 segregation for cross 9 was as follows:

	Bearded	Hooded and heterozygous	Total
Observed	91	267	358
Expected	89.5	268.5	358

$$\chi^2 = 0.0149 \quad \text{D.F.} = 1 \quad P = 0.90 \text{ approx.}$$

Hooded bearded *T. vulgare* therefore differs from bearded by a single gene. The results of crosses 10-13 and 17 reported below show that this gene is not allelomorphous with either B_1 or B_2 , so that it may be denoted by a fresh symbol **Hd**, hooded bearded being **Hd Hd**, and bearded **hd hd**.

(f) *Hooded bearded* \times *tipped 2*.

This segregation was studied in F_2 populations of crosses 10-13, the results of which are presented in Table III (expected values based on a 15:1 ratio are put in brackets throughout). Only the bearded segregates were accurately distinguishable in F_2 , the remaining plants containing hooded bearded, tipped 2, hooded beardless and various unclassifiable intermediate types.

The above 15:1 segregation shows that the "hooded" gene **Hd** and the tipped 2 gene B_2 are not allelomorphous. There is a slight deficiency of bearded plants in the above families, which may be due to pollen competition or to differential viability in the severe spring of 1938. The per-

centages of F_2 grains sown which produced mature plants in the above families were 47.2, 70.7, 59.9 and 65.9 respectively, so that the possibility of selective elimination certainly exists. Alternatively, the deficiency might have been due to a weak linkage between the genes **Hd** and **B₂**, since bearded is a recombination type in the above crosses; but this possibility is unlikely for two reasons: (1) the factors **Hd** and **B₂** show no significant linkage in crosses 14 and 15, and (2) a similar deficiency of bearded plants occurs in certain other crosses involving **B₂** where single-gene ratios were examined, viz. crosses 3 and 14.

The cross hooded bearded \times tipped 1 was not made, but the genes **Hd** and **B₁** are involved together in cross 17.

TABLE III

Segregation in hooded bearded \times tipped 2 crosses

Cross	Bearded	Others	Total	χ^2	Degrees of freedom
10	20 (14.25)	208 (213.75)	228	2.063	1
11	28 (40.6)	622 (609.4)	650	3.860	1
12	21 (26.69)	406 (400.31)	427	1.076	1
13	24 (34.1)	522 (511.9)	546	2.896	1
				9.895	4
Total	93 (115.7)	1758 (1735.3)	1851	4.539	1

Analysis of χ^2

	χ^2	D.F.	P
Deviation	4.539	1	0.02-0.05
Heterogeneity	5.356	3	0.10-0.20
Total	9.895	4	

(g) *Hooded beardless \times bearded.*

Cross 14, which involves hooded beardless *T. vulgare* and bearded *T. sphaerococcum*, is described at length elsewhere (Ellerton, 1939). Complete classification for awn type was only possible in the *vulgare* segregates, but it was shown that the hooded beardless type carries the genes **Hd** and **B₂**, which are independently inherited, so that one-sixteenth of the progeny were fully bearded. The F_2 figures, based on F_3 progeny tests, for these *vulgare* segregates were as follows:

	Hd Hd	Hd hd	hd hd
Observed	96	163	72
Expected	82½	165½	82½
$\chi^2 = 3.556$. D.F. = 2. $P = 0.10-0.20$.			
	B₂B₂	B₂b₂	b₂b₂
Observed	97	176	56
Expected	82½	164½	82½
$\chi^2 = 11.827$. D.F. = 2. $P < 0.01$.			

The F_2 contained hooded beardless, hooded bearded, tipped 2 and

262 *Variation and Genetics of the Awn in Triticum*

bearded forms in addition to the various expected intermediate heterozygotes. The F_3 results show a significant deficiency of b_2b_2 plants, a result similar to that shown in cross 3 and suggested in crosses 10-13. The deficiency was shown to occur equally in the **SS** (pure *vulgare*) and **Ss** (heterozygous *vulgare*/*sphaerococcum*) segregates, so that it cannot be due to linkage with the **s** factor.

The combination hooded beardless \times bearded was also studied in cross 15, which is an intergroup cross in which normal segregations were obtained by backcrossing the pentaploid F_1 to the parents. The segregations in this cross, based on progeny tests of the backcross plants, are shown in Table IV. Only the awn genes **B₂** and **Hd** and their linkage with **K** (keeled glume) are considered.

TABLE IV

Segregation of awn genes in Chinese White \times Rivet Backcrosses

(a) Single-gene ratios						
Combination	B₂ : b₂	Degrees of freedom	χ^2	Hd : hd	Degrees of freedom	χ^2
(C.W. \times Rivet) $F_1 \times$ C.W.	32 : 46	1	2.167	47 : 33	1	2.113
(Rivet \times C.W.) $F_1 \times$ Rivet	29 : 17	1	2.399	22 : 24	1	0.022
(C.W. \times Rivet) $F_1 \times$ Rivet	69 : 62	1	0.275	61 : 70	1	0.489
		3	4.841		3	2.624
Total	130 : 125	1	0.063	130 : 127	1	0.016
(b) Analysis of χ^2						
	χ^2	B₂ : b₂ Degrees of freedom	<i>P</i>	χ^2	Hd : hd Degrees of freedom	<i>P</i>
Deviation	0.063	1	0.80 approx.	0.016	1	0.90 approx.
Heterogeneity	4.778	2	0.05-0.10	2.608	2	0.20-0.30
Total	4.841	3		2.624	3	
(c) Linkage relations						
Gene pair	Parental combinations	Re-combinations	χ^2	<i>P</i>		
B₂ and Hd	135	120	0.769	0.30-0.50		
B₂ and K	94	102	0.250	0.50-0.70		
Hd and K	100	98	0.005	0.90-0.95		

The above results therefore confirm those of cross 14, Chinese White differing from bearded Rivet by two awn genes **Hd** and **B₂**. The genes **Hd** and **B₂** do not show any significant linkage with each other, and it also appears that both genes probably segregate independently of the gene **K** for keeled glumes. The absence of linkage between **Hd** and **B₂** is further shown in cross 14 above.

The three different backcross families in the above table do not show significantly different segregations for **Hd** or **B₂**. With respect to **B₂**, however, the value of χ^2 for heterogeneity approaches significance and there is a suggestion that there is again a disturbance due to a deficiency of **b₂b₂** plants. The backcrosses to Rivet, in which the ratio should be 1 **B₂b₂** : 1 **b₂b₂**, actually gave a total of 98 **B₂b₂** : 79 **b₂b₂**. This is not a significant deviation from a 1 : 1 ratio, but it certainly cannot be asserted, on the basis of these data, that the deficiency of **b₂b₂** plants found in cross 14 does not also occur in the present cross. The loss may have been due to differential viability of the young plants, since by no means all of the seeds sown in this cross produced mature plants.

(h) *Hooded beardless* \times *tipped 2*.

Cross 16 showed only hooded beardless and tipped 2 segregates in F_2 , together with the expected intermediate heterozygotes. The ratio appeared to be roughly 1 : 2 : 1, but accurate classification proved impossible. The absence of bearded or hooded bearded segregates in this progeny shows conclusively that the tip-awned gene carried by Chinese White is actually **B₂**, a fact that had already been deduced from the phenotypic appearance of the tip-awned segregates in crosses 14 and 15 above.

(j) *Hooded beardless* \times *tipped 1*.

The behaviour of cross 17, Chinese White \times Squarehead's Master, may now be predicted. Chinese White has the constitution **b₁b₁B₂B₂Hd Hd**, while Squarehead's Master is **B₁B₁b₂b₂hd hd**. One would therefore expect one sixty-fourth of the progeny to be fully bearded, the remaining types including hooded bearded, tipped 1, tipped 2, hooded beardless and ordinary beardless wheats together with all possible intermediate heterozygotes. This was actually the case. In F_2 only the fully bearded segregates could accurately be distinguished, the segregation being as follows:

	Bearded	All others	Total
Observed	13	1095	1108
Expected	17.3	1090.7	1108

$$\chi^2 = 0.853. \quad \text{D.F.} = 1. \quad P = 0.30-0.50.$$

The result is a good fit to the expected 1 : 63 ratio.

(k) *Beardless* \times *bearded*.

The only cross in which this combination was studied, cross 18, was an intergroup cross in which inheritance proved to be very complicated owing to irregularities not yet properly understood. The results served to show, however, that the beardless parent, Australia 6, differed from

264 *Variation and Genetics of the Awn in Triticum*

bearded in two genes, which separately determined the tipped 1 and half-awned condition. Bearded, half-awned, tipped 1 and beardless pure-breeding *durum* segregates were isolated. The beardless *durum* plants were stunted and showed very considerable sterility, even after several generations of selection.

(4) *The hooded character of Abyssinian tetraploids*

The cross bearded *durum* × hooded bearded Abyssinian tetraploid showed high fertility, and no genetic irregularity was observed in the inheritance of the hooded character.

The F_1 and the heterozygous F_2 plants resembled the bearded parent quite closely in their main tillers but had markedly hooded late tillers. It should therefore have been possible ideally to effect a complete classification of the F_2 . This was impossible in practice, however, owing to the fact that many plants had single tillers or two nearly equal tillers only, so that they could not be classified definitely as either heterozygous or bearded. The F_2 classification was therefore made for the pure hooded plants *v.* all others. The result was:

	Hooded	Bearded and heterozygous
Observed	113	362
Expected	118½	356½

$$\chi^2 = 0.070. \quad P = 0.70-0.80.$$

All the F_2 plants that yielded enough grain to plant a row were grown on to F_3 , where the F_2 classification was found to be substantially correct. The F_3 ratio for 415 F_2 plants which had been classified as 79 hooded : 236 bearded and heterozygous, was 77 : 238. Three heterozygous plants had been wrongly classed as pure hooded, and one hooded plant had been classed as heterozygous in F_2 . The single-gene inheritance of the hooded character was therefore fully confirmed in F_3 .

The Abyssinian parent in this cross had considerably shorter straw than the bearded parent, so that measurements were made of the plant height of the parents, the F_2 , and a random sample of F_3 rows, to determine whether there was any linkage between short straw and hoods. The results are expressed in Table V; the parent rows measured were growing next to the F_2 on an apparently uniform plot, so that the heights quoted may be regarded as comparable.

It may be concluded from the above results that the hooded factor in Hooded Abyssinian is linked to a factor which reduces length of straw (the actual distance measured was from the rooting node to the tip of the ear, excluding the awns). The difference between the heights of the parents

(33.5 cm.) was, however, much greater than that between the hooded segregates and the other two groups (11 cm.). It therefore appears likely that the parents differed by genes other than the one that is linked with the hooded gene, and that these segregate independently. This possibility is confirmed by the measurements of heights of individual families in F_3 , which showed clear differences due to the segregation of other height genes.

The closeness of the linkage between the hooded gene and the gene determining short straw cannot be determined without extensive biometrical studies, which were not carried out. The F_3 results suggested, but did not prove, that the linkage was not absolute. The mean plant

TABLE V
Relation between hooded and plant height in the cross
Hooded Abyssinian \times *Indian Runner*

	Plant height in cm.
Parents:	
Indian Runner	102 \pm 2.2
Hooded Abyssinian	69 \pm 1.6
Difference	33 \pm 2.7
F_2 :	
Bearded and heterozygous plants	104 \pm 0.7
Hooded	92 \pm 1.2
Difference	11 \pm 1.4
F_3 :	
17 pure bearded families	86
24 segregating families	87
17 pure hooded families	75
Difference between hooded families and others ca. 11 cm.	

heights in centimetres for seventeen hooded families were as follows: 99.6, 76.2, 82.9, 77.7, 67.8, 73.5, 73.3, 65.6, 65.9, 79.3, 90.3, 70.8, 71.4, 74.1, 79.1, 82.4, 74.0. The average standard error of these figures is about ± 3.75 cm. The two families with mean heights of 99.6 and 90.3 show a fairly marked discontinuity from the others, and compare with mean heights of 74.3 for the remaining hooded families and 86.2 for the bearded families. These two may therefore have been cross-overs between the hooded gene and the short straw gene to which it is linked.

VI. DISCUSSION

With these results and those of previous workers we are now able to make a comprehensive statement of the mode of inheritance of awns in *T. vulgare*, as regards the major awn types. Bearded forms must be regarded as the bottom recessive in each case. There are five established

266 *Variation and Genetics of the Awn in Triticum*

major genes which, alone or in combination, lead to the production of the major awn classes. These, together with the phenotypes they produce when acting alone, may be denoted as follows:

B₁	Tipped 1
b₁^a	Half-awned (allelomorphic with B₁ and b₁)
B₂	Tipped 2
A	Half-awned
Hd	Hooded

In addition, it is possible that the rare half-awned types with awns of practically equal length in all parts of the ear may be due to another gene—they were not studied in the present crosses and have not been reported upon elsewhere.

On the basis of the above five genes, the constitution of the awn types of *T. vulgare* may be represented as follows:

Bearded	b₁b₁b₂b₂aa	hd hd	
Tipped 1	B₁B₁b₂b₂aa	hd hd	
Tipped 2	b₁b₁B₂B₂aa	hd hd	
Half-awned	b₁^ab₁^ab₂b₂aa	hd hd	or
	b₁b₁b₂b₂AA	hd hd	
Beardless	B₁B₁B₂B₂aa	hd hd	or
	B₁B₁b₂b₂AA	hd hd	
Hooded bearded	b₁b₁b₂b₂aa	Hd Hd	
Hooded beardless	b₁b₁B₂B₂aa	Hd Hd	

The above types are those actually shown to exist. In addition, various other combinations would be expected to give the beardless and hooded beardless types, e.g. **b₁b₁B₂B₂AA hd hd** in the former case, and **B₁B₁b₂b₂aa Hd Hd** in the latter.

The only possible simplification of the above scheme is that the genes **B₂**, **A** and **b₂** may actually be multiple allelomorphs, as are the genes **B₁**, **b₁^a** and **b₁**. No cross involving **A** and **B₂** has been studied genetically but that this multiple allelomorph relationship may actually hold is suggested by three things. First, one may expect a parallel series to the **B₁**, **b₁^a**, **b₁** series to be present in hexaploid wheats, on the basis of the law of homologous series in variation. Secondly, it is curious that all the beardless wheats containing **A** which have been studied also contain **B₁** and not **B₂**, and this in spite of the fact that several of them were of Indian origin, and the **B₂** gene is ten times as common in India as **B₁**. This strongly suggests that a wheat of the constitution **B₂B₂AA** cannot exist, because **B₂** and **A** are homologous. Thirdly, although a complete classification of half-awned wheats into subtypes was not possible, it seems to be true that those having the gene **b₁^a** have awns more definitely localized at the apex of the ear than those carrying the gene **A**. In other words, the difference between these two types corresponds to that between tipped 1 and tipped 2 wheats. If the genes **B₂**, **A** and **b₂** are actually multiple allelomorphs,

the series would be represented as B_2 , b_2^a , b_2 , and the genetic constitution of the above types would be:

Bearded	$b_1b_1b_2b_2$	$hd\ hd$	
Tipped 1	$B_1B_1b_2b_2$	$hd\ hd$	
Tipped 2	$b_1b_1B_2B_2$	$hd\ hd$	
Half-awned	$b_1^ab_1^ab_2b_2$	$hd\ hd$	or
	$b_1b_1b_2^ab_2^a$	$hd\ hd$	
Beardless	$B_1B_1B_2B_2$	$hd\ hd$	or
	$B_1B_1b_2^ab_2^a$	$hd\ hd$	or (presumably)
	$b_1^ab_1^aB_2B_2$	$hd\ hd$	
Hooded bearded	$b_1b_1b_2b_2$	$Hd\ Hd$	
Hooded beardless	$b_1b_1B_2B_2$	$Hd\ Hd$	(or, presumably, Hd with b_2^a , B_1 or b_1^a)

The latter system is regarded by the writers as being the more probable. The crucial cross necessary to distinguish between the two schemes will be made at the earliest opportunity.

In addition to the above, we have certain other information about these genes. B_1 is linked with the genes for pubescent node, square-headedness and keeled glumes (speltoid). The genes B_1 , B_2 , and Hd may all be transferred to tetraploid wheats and there segregate regularly. None of them appears to be linked with any other in normal wheats, except perhaps in the case of the unusual linkage between B_1 and A recorded by Stewart (1926, 1928 b) in two Federation crosses.

As regards the Abyssinian wheats, only the hooded gene was studied. This has a similar effect to the gene Hd found in *T. vulgare* and has the same curious dominance relations. The cross between hooded Abyssinian and a hooded *vulgare* wheat has now been made to establish the identity of the two genes, but it seems probable that the same gene is involved in each case.

With an accurate classification of the awn types occurring in wheats and a clear view of their mode of inheritance, it will be possible to consider their geographical distribution in Eurasia. This will be dealt with in a later paper.

SUMMARY

1. Awn length in *Triticum* is affected by both major and modifying genes, as well as by chromosome number, hexaploids usually having shorter awns than tetraploids.

2. Most tetraploid wheats are fully awned, but a number of other types are found in Abyssinia. One of these, the hooded type, was shown to differ from fully bearded by a single gene.

3. There is a wide variation among hexaploids, and the following main genes have been established:

B₁, the gene for tipped 1, reduces the awn so that only a few awn tips, up to 1-2 cm. occur; the longest tips are found towards the top of the ear.

b₁^a forms a multiple allelomorph series with **B₁** and **b₁**, the gene for bearded, and gives "half-awned" types with short awns.

B₂, the gene for tipped 2, reduces the awn to a few short tips which are usually of approximately equal length from top to bottom of the ear; crosses involving **B₂** and **b₂** usually show a deficiency of **b₂b₂** segregates.

A, a second gene for half-awned, may form a multiple allelomorph series with **B₂** and **b₂** but this is not yet proved.

Hd reduces the length of the awns, and generally makes them curved and twisted near the base; the effect is greatest on late tillers.

Bearded wheats are therefore **b₁b₁ b₂b₂ hd hd**, tipped 1 is **B₁B₁ b₂b₂ hd hd**, tipped 2 is **b₁b₁ B₂B₂ hd hd**, hooded is **b₁b₁ b₂b₂ Hd Hd**, beardless is **B₁B₁ B₂B₂ hd hd**, hooded beardless is **b₁b₁ B₂B₂ HdHd**, and various other types are possible.

4. Twenty standard lines of known genetical composition have been established.

REFERENCES

- BIFFEN, R. H. (1905). "Mendel's laws of inheritance and wheat breeding." *J. agric. Sci.* **1**, 4-48.
- CLARK, J. A. (1924). "Segregation and correlated inheritance in crosses between Kota and Hard Federation wheats for rust and drought resistance." *J. agric. Res.* **29**, 1-47.
- CLARK, J. A., FLORELL, V. H. & HOOKER, J. R. (1928). "Inheritance of awnedness, yield and quality in crosses between Bobs, Hard Federation and Propo wheats at Davis, California." *Tech. Bull. U.S. Dep. Agric.* no. 39, pp. 39.
- CLARK, J. A. & HOOKER, J. R. (1926). "Segregation and correlated inheritance in Marquis and Hard Federation crosses with factors for yield and quality of spring wheat in Montana." *Bull. U.S. Dept. Agric.* no. 1403, pp. 70.
- CLARK, J. A. & QUISENBERRY, K. S. (1929). "Inheritance of yield and protein content in crosses of Marquis and Kota spring wheats grown in Montana." *J. Agric. Res.* **38**, 205-17.
- ELLERTON, S. (1939). "The origin and geographical distribution of *Triticum sphaerococcum* Perc. and its cytogenetical behaviour in crosses with *T. vulgare* Vill." *J. Genet.* **38**, 307-24.
- FLAKSBERGER, C. (1930). "Ursprungszentrum und geographische Verbreitung des Spelzes (*Triticum Spelta* L.)." *Angew. Bot.* **12**, 86-99.
- GAINES, E. F. (1917). "Inheritance in wheat, barley and oat hybrids." *Bull. Wash. St. agric. Exp. Sta.* no. 135, 3-61.
- GAINES, E. F. & CARSTENS, A. (1926). "The linkage of pubescent node and beard factors as evidenced by a cross between two varieties of wheat." *J. agric. Res.* **33**, 753-55.

- GAINES, E. F. & SINGLETON, H. P. (1926). "Genetics of Marquis \times Turkey wheat in respect to bunt resistance, winter habit and awnlessness." *J. agric. Res.* **32**, 165-81.
- HOSONO, S. (1934). "Distribution and spread of wheat in China." *Kagaku (Science), Tokyo*, **4**, 509-12.
- HOWARD, A. & HOWARD, G. L. C. (1912). "On the inheritance of some characters in wheat. I." *Mem. Dep. Agric. India, Bot.* **5**, 1-47.
- (1915). "On the inheritance of some characters in wheat. II." *Mem. Dep. Agric. India, Bot.* **7**, 273-85.
- JENKIN, T. J. (1925). "Natural crossing in wheat." *Welsh J. Agric.* **1**, 104-10.
- KAJANUS, B. (1923). "Genetische Untersuchungen an Weizen." *Biblioth. genet.* **5**, 1-187.
- (1927). "Ueber einige Fälle erheblicher Abweichung in habituell zweigliedrigen Spaltungen bezüglich der Begrannung bei Weizen." *Hereditas, Lund*, **9**, 25-32.
- KOBELEV, V. K. (1928). "General conditions of cultivation of wheat in Afghanistan." *Bull. appl. Bot., Leningrad* **19** (1), 3-120.
- LOVE, H. H. & CRAIG, W. T. (1924). "The inheritance of pubescent nodes in a cross between two varieties of wheat." *J. agric. Res.* **28**, 841-44.
- (1926). "The genetics of Sonora wheat." *J. Amer. Soc. Agron.* **20**, 307.
- MATSUMURA, S. (1936). "Genetische Studien über die pentaploiden Weizenbastarde. II. Vererbung der von den Chromosomenzahlen unabhängigen morphologischen Eigenschaften bei den Verbindungen *Triticum polonicum* \times *T. Spelta*." *Jap. J. Genet.* **12**, 289-306.
- MATSUURA, H. (1933). *A bibliographical monograph on plant genetics (Genic analysis)*, 1900-29. Hokkaido University.
- NILSSON-EHLE, H. (1920). "Multiple Allelomorphe und Komplexmutationen beim Weizen (Untersuchungen über Speltoidmutationen beim Weizen. II)." *Hereditas, Lund*, **1**, 277-311.
- (1927). "Das Verhalten partieller Speltoidmutationen bei Kreuzung untereinander (Untersuchungen über Speltoidmutationen beim Weizen. IV)." *Hereditas, Lund*, **9**, 369-79.
- NILSSON-LEISSNER, G. (1925). "Beiträge zur Genetik von *Triticum Spelta* und *Triticum vulgare*. I." *Hereditas, Lund*, **7**, 1-74.
- PERCIVAL, J. (1921). *The Wheat Plant. A Monograph*. London.
- PHILIPTSCHENKO, J. A. (1934). *Genetics of Soft Wheat*. Moscow.
- QUISENBERRY, K. S. & CLARK, J. A. (1933). "Inheritance of awn development in Sonora wheat crosses." *J. Amer. Soc. Agron.* **25**, 482-92.
- STEWART, G. (1926). "Correlated inheritance in wheat." *J. agric. Res.* **33**, 1163-92.
- (1928 a). "Correlated inheritance in Kanred \times Sevier varieties of wheat." *J. agric. Res.* **36**, 873-96.
- (1928 b). "Inheritance of awns in crosses involving Sevier and Federation wheat." *J. Amer. Soc. Agron.* **20**, 160-70.
- STEWART, G. & BISCHOFF, R. K. (1931). "Correlated inheritance in a cross (Sevier \times Dicklow) \times Dicklow wheats." *J. agric. Res.* **42**, 775-90.
- STEWART, G. & HEYWOOD, D. E. (1929). "Correlated inheritance in a wheat cross between Federation and a hybrid of Sevier \times Dicklow." *J. agric. Res.* **39**, 367-92.

270 Variation and Genetics of the Awn in Triticum

- STEWART, G. & JUDD, B. I. (1931). "Inheritance of awns in a Kota \times Hard Federation cross." *J. Amer. Soc. Agron.* **23**, 455-64.
- STEWART, G. & TINGEY, D. C. (1928). "Transgressive and normal segregations in a cross of Marquis \times Federation wheats." *J. Amer. Soc. Agron.* **20**, 620-34.
- VAVILOV, N. I. (1923). "A contribution to the classification of soft wheats—*Triticum vulgare* Vill." *Bull. appl. Bot. Leningrad*, **13**, 149-257.
- VAVILOV, N. I. & BUKINICH, D. D. (1929). "Agricultural Afghanistan." *Bull. appl. Bot. Leningrad*, Suppl. **33**, 1-610.
- VAVILOV, N. I. *et al.* (1931). ["The wheats of Abyssinia and their position in the general system of the wheats. (Contribution to the knowledge of the 28-chromosome group of cultivated wheats)."] *Bull. appl. Bot. Leningrad*, Suppl. **51**, 1-236.
- WATKINS, A. E. (1928). "The genetics of wheat species crosses. I." *J. Genet.* **20**, 1-27.
- (1940). "The inheritance of glume shape in *Triticum*." *J. Genet.* **39**, 249-64.
- WATKINS, A. E. & CORY, F. M. (1931). "Genetic and cytological studies in wheat. V." *J. Genet.* **25**, 55-90.

EXPLANATION OF PLATES X—XII

PLATE X

Fully bearded hexaploid wheats.

- A, B, D. Fully bearded *T. vulgare* with exceptionally long, exceptionally short and average awn length respectively. C. Fully bearded *T. sphaerococcum* showing the reduction in awn length caused by the *sphaerococcum* gene, *s*. (All $\times \frac{2}{3}$.)

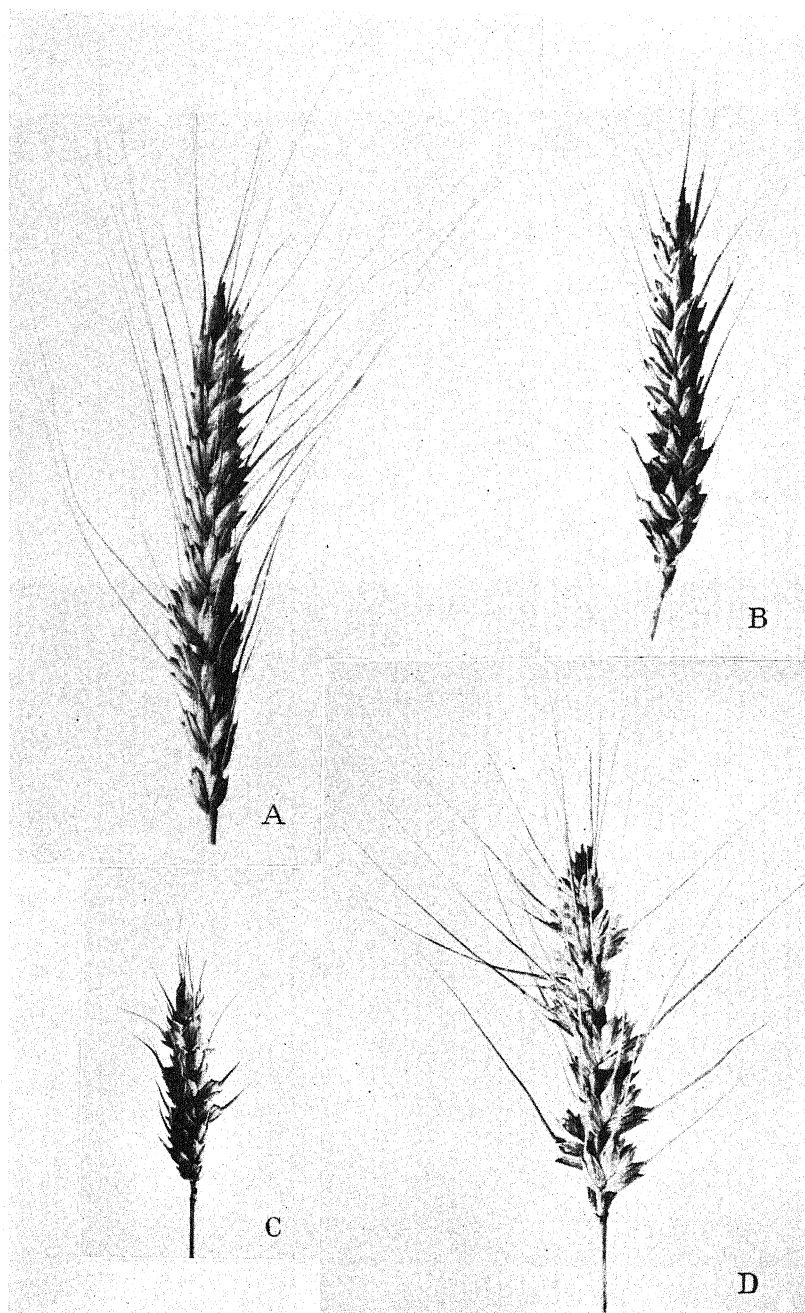
PLATE XI

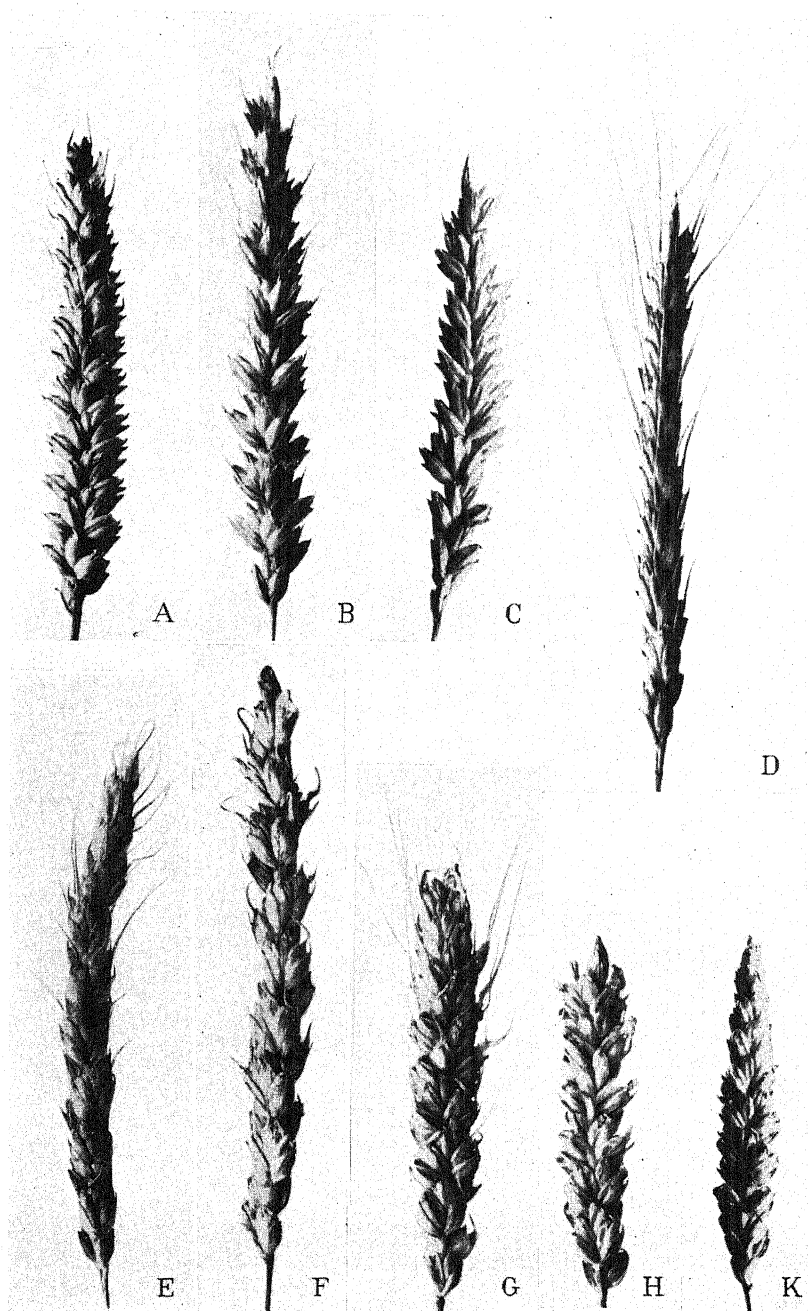
Awn types in *T. vulgare*.

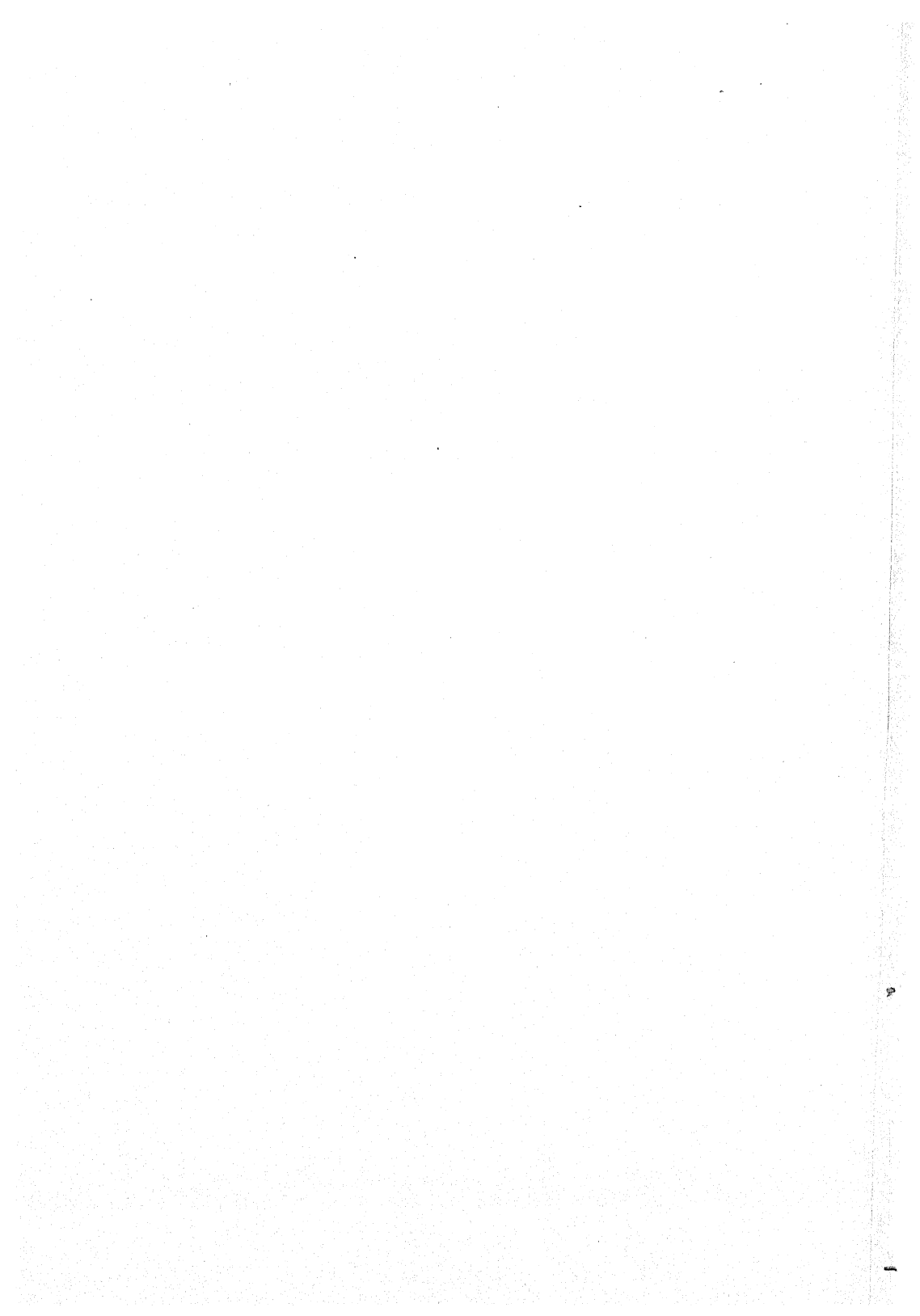
- A. Tipped 1 (apically tip-awned). B. Tipped 2 (tip-awns evenly distributed). C. Beardless. D. Half-awned. E, F, G. Hooded bearded (main tillers of different wheats). H. Late tiller from the same hooded bearded plant as G. K. Hooded beardless. (All $\times \frac{2}{3}$.)

PLATE XII

The hooded bearded character in Abyssinian tetraploid wheat (a main tiller and late tiller from the same plant, both $1\frac{1}{2}$ times natural size).









THE APPLICATION OF GENETICS TO PLANT BREEDING

I. THE GENETIC INTERPRETATION OF PLANT BREEDING PROBLEMS¹

By J. B. HUTCHINSON

*Empire Cotton Growing Corporation, Cotton Research
Station, Trinidad, B.W.I.*

CONTENTS

	PAGE
I. Introduction	271
II. Variability and the choice of material in which to select	272
III. The improvement of the efficiency of selection	276
IV. Discussion	280
References	282

I. INTRODUCTION

THE influence of the science of genetics on the art of plant breeding has been much less profound than was expected by the early geneticists. For the elucidation of the mechanism of heredity, only factors causing large differences were useful, and it was not immediately realized that these are of little importance in nature or under artificial selection. Progress in the genetic control of small differences has been difficult and slow and many geneticists still consider that minor genes are not worth investigating on their own account. Some genetic principles have been successfully applied by breeders, but often the principles involved are not fully realized, and the application has usually been made with no experimental support beyond deductions from general observations on breeding material. In correspondence on breeding methods it is remarkable how often a practice of fundamental importance is justified by a statement beginning "My feeling is. . . ." It should be the function of the geneticist to substitute objective evidence for the breeder's intuition.

It is the object of this paper to survey the application of genetic theory to cotton breeding in particular and to outline the problems in which the geneticist should be able to help the breeder.

¹ Read at the Seventh International Genetic Congress, Edinburgh, August 1939.

II. VARIABILITY AND THE CHOICE OF MATERIAL IN WHICH TO SELECT

Until recently it was generally accepted that the action of natural selection resulted in the purification and propagation of the fittest type in a population. A perfectly adapted type would theoretically be pure, and the existence of variability was usually supposed to indicate recent crossing between pure types (Patel, 1920; Stock, 1927). Recent work on crop populations has shown that the effect of natural selection on variability is very different from what had been supposed. Hutchinson and others (1937 *a*, 1938 *b*) as a result of studies on crop populations in India, concluded that natural selection results in the establishment of a well-adapted population consisting of a great range of types. When grown in pure culture these types differ so much in yield that it is necessary to postulate considerable competition effects to explain their survival in the mixed crop. In most cases the nature of the forces maintaining variability is obscure, but some general observations can be made on such data as are now available. In Central India, American Upland cotton has become established as a permanent component of the cotton crop. In pure culture the indigenous types give a considerably heavier yield. In mixed crops the Upland type gains by competition. The disparity in yield per plant is reduced, and the germination of the Upland is much better than that of the indigenous types. The mixed product is of better quality than the pure indigenous cotton and is preferred by the local mills, and seed from mixtures with the highest proportion of Upland is returned to the cultivators. The lower intrinsic yield of the Upland type is balanced, therefore, by advantages in competition, germinating capacity and quality. In many cases, selective forces vary from place to place in the ecological area. For example, in Central India, cultivators select and grow either yellow grained or white grained *jowar* (*Sorghum durra*) according to personal preference. Selective forces are also likely to vary from season to season as well as from place to place in comparatively small areas, and the effect of such changes in maintaining variability is obvious. It may be concluded that a genetically variable strain will give a more flexible response to environmental variations than a very uniform one. If variability, and not uniformity, is the natural characteristic of crop populations, the breeder's ideal must be reconsidered. Selection for uniformity should not proceed beyond the stage needed to ensure a satisfactory grade in the marketable product, except where it is possible by further specialization to achieve closer adaptation to local climates. Where seasonal fluctuations are of most importance, as for instance in

Tanganyika and Queensland, the maintenance of some genetic variability is a definite item in breeding policy. In the Sudan, on the other hand, place to place variations are such that progress has been made by breeding special strains for different localities (King, 1938). The U 4 variety of Upland cotton in its original variable form proved well adapted to a wide range of conditions in Africa, and selection work has been carried out on it at a number of centres. Local adaptation has become so pronounced that there is now little exchange of substrains between breeding stations and such material as has been exchanged has failed to compete with local selections (Peat & Prentice, 1939).

In early line breeding work it was assumed that the ideal was a "pure line", and once this was established all that was necessary was to preserve it from contamination by mixing or hybridization and it would remain pure indefinitely. Experience with Sea Island cotton in the West Indies shows that this ideal is in practice unattainable. The rate of mutation in certain major genes is high enough to cause serious contamination in a comparatively short time. General deterioration in quality occurs unless there is a constant renewal of the seed supply from selected stocks, even in islands where no cottons exist save the single pure strain under cultivation. The superfine Sea Island strains are very different from what would have arisen in nature, and in the absence of selective breeding, natural selection rapidly changes the strain characteristics.

All cotton breeding experience supports the view that variability is the natural state of unselected populations, and persists in some measure in even the most closely bred pedigree stocks. Degeneration has been most rapid and most serious in the best and most closely bred varieties. Unselected variable "land races", on the other hand, maintain their characteristics unchanged indefinitely. It is a fair inference that the behaviour of a strain depends on selection pressure and not on variability, and the effort at present devoted to achieving purity may profitably be used to increase the efficiency of selection.

Vavilov (1935) and other Russian botanists have studied the distribution of variability in cultivated crops and have postulated a limited number of comparatively small areas where most of the world's crops have been evolved. In these areas, which are usually mountainous and therefore divided into many comparatively isolated pockets, they found that variability is extremely high, and it diminished, usually by the progressive loss of dominant genes, from the centre of origin to the periphery of the distribution. In areas where a species has undergone rapid multi-

plication and extensive distribution secondary centres of variability may arise. In cotton the situation is not always as simple as in the cases quoted by Vavilov (Hutchinson, 1938 *a*). The two cultivated American species have their centres of variability in the two centres of crop variability postulated by Vavilov (1935) in Central America and in the Cordillera-Andean ranges of Colombia, Ecuador and Peru. Of the cultivated species of the Old World, *Gossypium herbaceum* appears to have its primary (though less important) centre of variability in the savannah countries of West Africa, with secondary centres more important than the primary one in Western India and in Iran, Afghanistan and Russian Turkestan. In *G. arboreum* there are at least four centres of variability, in the Sudan, South India, Assam and East Bengal, and eastern Burma and Indo-China. The discovery that most of the variability in a species is to be found in a small fraction of its total range, is of great value both in interpreting past breeding problems and in guiding the choice of breeding material. The value of material from areas of high variability has been stressed chiefly by workers in countries outside those areas, and they have built up large collections of types as reserves for future breeding work. Recently there has been a tendency for countries such as India where the variability is great to follow the Russian example. It should be obvious that no collection of types can compete with the untouched native variability of the cultivator's field, and where there are rich indigenous variable crop populations it is more important to see that they are maintained than to build up large type collections.

Recently Mason (1938) has commented on the lack of success in synthesizing new strains of cotton from hybrid material. In India, to which Mason's remarks particularly referred, there are two centres of variability of *G. arboreum* and one of *G. herbaceum*, and it is therefore natural that the exploitation of existing variability has taken precedence over selection in hybrid material. The United States cotton belt, on the other hand, is outside the area of high variability in *G. hirsutum*, and the variability required for breeding work has been more frequently found in hybrid stocks. In Egypt also, where *G. barbadense* is not indigenous, hybrid material has been found to be the best source of variability (Brown, 1939).

Apart from the high variability of unselected local cotton crops, the use of hybridization as a source of variable material has been discouraged in India by repeated failure. The causes of failure have been elucidated by Harland's (1936) analysis of the genetic nature of species differences and the revision of the classification of the genus in the light of genetic

results (Harland, 1932; Hutchinson & Ghose, 1937 *b*). Harland's work was chiefly with the two New World cultivated species, *G. hirsutum* and *G. barbadense*. He showed that though they cross freely and give a very vigorous F_1 , homologous characters are built up in such widely different ways that the genetic balance is disintegrated by segregation in F_2 and later generations. Vigorous and productive selections are almost invariably multiple heterozygotes which segregate into a welter of types, the majority of which are inferior in vigour and productivity and often morphologically abnormal. Harland (1936) further showed that it was possible to transfer single genes or small groups of genes from one species to another without disturbing the species balance. He concluded that improvement by interspecific hybridization is to be looked for through the addition of small groups of genes from one species to the complement of the other, and not through breeding intermediate types. The revision of the classification of the cottons of the Old World by Hutchinson & Ghose (1937 *b*) made it possible to examine hybridization experiments with Indian cottons in the light of Harland's theory of species balance. It was found that those crosses with which success had been achieved were between types regarded on genetic grounds as members of a single species (*G. arboreum*). Those from which no satisfactory types had been isolated were from crosses between *G. arboreum* and *G. herbaceum*, which are genetically distinct species. In breeding work with such hybrids, vigorous productive selections were shown to be multiple heterozygotes which broke down into a range of unproductive, often unbalanced types. The conclusions drawn from experimental hybrids were confirmed by field studies of crops in which the two species are normally grown mixed. Although hybridization occurs and F_1 's, by reason of their hybrid vigour, are the outstanding plants in the crop, later generation hybrids are rare or absent, and there is no tendency whatever for the species distinction to disappear.

No breakdown has been observed in intervarietal or inter-strain crosses, though intervarietal diversity in the genetic basis of important lint characters has been demonstrated by Silow (1939). Hybrid material that does not transgress species boundaries can be confidently recommended to breeders for straight selection. Among the Asiatic cottons two of the most promising lines of improvement in *G. arboreum* are selections from hybrids between northern Indian and Chinese forms and between northern and southern Indian forms. These have been shown (Hutchinson & Ghose, 1937 *b*; Hutchinson *et al.* 1938 *c*) to involve differences of approximately varietal rank. Among New World cottons, hybrids of

G. hirsutum × *G. hirsutum* var. *religiosum* are likely to be of value for selection under African conditions.

With the understanding that success will be dependent upon rapid re-establishment of the genetic balance of one or other species, the plant breeder may now return with some confidence to interspecies hybridization. Considerable success has already been achieved by Knight & Clouston (1939) in transferring the "blackarm" (*Bacterium malvacearum*) resistance of a strain of *G. hirsutum* to an Egyptian type of *G. barbadense*. They backcrossed the interspecies F_1 three times to the Egyptian parent, selecting rigorously in each segregating generation types with F_1 resistance and as nearly as possible the morphological characteristics of the Egyptian parent.

III. THE IMPROVEMENT OF THE EFFICIENCY OF SELECTION

In the earliest days of cotton breeding Balls (1919) emphasized the importance of the components of yield. Through his work in Egypt and Harland's (1920) in the West Indies, it has become an accepted practice among breeders to study such components of yield as bolls per plant, seed cotton per boll, seeds per boll, lint per seed, etc. It soon became evident that the effects of environmental fluctuation were much greater on some characters (e.g. bolls per plant) than on others (e.g. seed cotton per boll) and it followed that selection on the latter group is much more effective than on the former. Improvement of the comparatively stable components of yield results in improvement in yield up to a point. Beyond that point the gains due to selection are compensated by depreciation in characters beyond the reach of selection. In the Heaton strain of Sea Island cotton lint index (weight of lint on 100 seeds) has been raised 30 % or more by selection and the greater part of the improvement is reflected in increased yield. Selection in the same strain for high weight of seed cotton per boll has given an improvement which is not reflected in a higher yield. Similar selection in an Upland cotton has resulted in a very large boll, and a disproportionate reduction in the number of bolls per plant. The common experience that intensification in one character can only be obtained at the expense of others is often the result of physiological incompatibilities. Even when there is no physiological barrier to combination, the chance of getting the maximum expression of character *B* in a plant selected for intensity of expression of character *A* is very small. Until the relative importance of these two possibilities is known no estimate can be made of the probable limits to improvement by selection. What is now required is a detailed physio-

logical-genetic analysis of the interrelations of the component characters of the plant. The organism is an integrated whole and it is obvious that any large change in one character must either be accompanied by corresponding changes in other characters, or disturb the balance of the plant. Change under selection should therefore be co-ordinated change, and not merely intensification of a single character.

Variation is of two kinds, environmental and genetic, and selection will be most efficient when the ratio of the genetic component to the total variance is at a maximum. The first great improvement in selection efficiency was the substitution of selection in progeny rows for mass selection. This is generally regarded as an application of the pure line theory, but its success is due to the fact that selection is more efficient when based on progeny means than on single plants. Between progenies the environmental component of the variance is less, and the genotypic component more than between plants.

Later attempts to improve the efficiency of selection have been concentrated on the problem of reducing the environmental variance in breeding material. In the "Purity Chequer" used in Egypt (Brown, 1932) environmental effects are reduced by replication. The plots are arranged systematically and every effort is made to provide optimum conditions for growth and development. No yield comparisons are made, but the seed cotton is examined for lint and seed characters. The data are analysed by correlation tables ("target diagrams"), and selections are made in progenies that combine high mean values and high variances in desirable characters. The weakness of the Egyptian system is that there is no attempt to evaluate yield characteristics in the early stages. Another systematic arrangement is that devised by Trought (1934) in which a line of the strain under test is compared with two lines of control, one on either side. As each comparison involves one line of the strain, two of the control and two guard rows, only one-fifth of the total area used is occupied by the material under test. Yield comparisons are made on a single plant basis with elaborate precautions to ensure that plants are only compared with exactly similarly spaced plants in the control rows.

Experimental designs involving randomization as well as replication were applied to progeny row breeding material by Hutchinson & Panse (1937 c), and have proved very successful in reducing the environmental contribution to the variance. The long delay in adopting modern statistical methods was probably due to the belief that a satisfactorily low error could not be expected with small plots. It does not appear to

have been realized that the very fact that progeny row selection has been successful shows that the differences between progenies are in general greater than can be ascribed to chance environmental fluctuations. It was therefore to be expected that the equalization of the major environmental differences by replication and arrangement in blocks would result in improved efficiency, even though very small plots were employed, while randomization to allow of the calculation of an estimate of the error of the progeny comparisons is obviously desirable.

TABLE I
*Standard errors in randomized block experiments with
breeding material of Montserrat Sea Island cotton*

Column ...	1		2		3		4	
	Experimental error expressed as coefficient of variation		Experimental error including block variation %		Block efficiency		Standard error of any strain mean %	
	Progenies	Bulks	Progenies	Bulks	Progenies	Bulks	Progenies	Bulks
Germination	23.8	—	24.8	—	1.09	—	7.53	—
Final stand	—	5.8	—	7.9	—	2.26	—	2.37
Node number	5.3	—	7.0	—	1.80	—	1.67	—
Days to first flower	3.2	—	4.6	—	2.04	—	1.42	—
Boll loculi	3.4	—	3.5	—	1.06	—	1.07	—
Bolls per plant	18.0	12.0	21.9	14.7	1.48	1.50	5.69	4.98
Seed cotton per boll	6.8	2.1	7.1	3.8	1.10	3.23	2.15	0.86
Seed weight	3.8	2.4	4.6	3.4	1.46	1.95	1.20	0.98
Lint index	4.9	3.3	6.4	4.3	1.69	1.75	1.55	1.35
Ginning %	4.3	2.7	4.8	3.5	1.26	1.73	1.36	1.10
Lint length	1.9	1.1	2.6	1.6	1.80	2.34	0.60	0.45
Yield of seed cotton	—	9.8	—	15.7	—	2.56	—	4.00
Yield of lint	—	10.0	—	14.6	—	2.15	—	4.08

The efficiency of selection in randomized block layouts with cotton may be illustrated from a progeny row trial and a small bulk trial of M.S.I. cotton in Montserrat in 1938. In the progeny row trial there were twenty single plant progenies in an experiment with ten blocks divided into five plant plots. In the small bulk trial there were twenty single progeny bulks (i.e. second generation from single plants) in an experiment with six blocks in which the plot size was 72 hills with one to two plants per hill. The efficiency of the experiments for each of the characters studied is summarized below (Table I). In the first column is given the coefficient of variation, evaluated by expressing the square root of the error variance as a percentage of the grand mean. Column 2 contains an estimate of the experimental error when the randomized block layout is ignored and the estimate of the error variance includes the effects of

fertility differences between the blocks. This error variance was calculated from the sum of squares for blocks + error, divided by the appropriate degrees of freedom. The square root of the resulting variance is given as a percentage of the grand mean. The best estimate of the efficiency of the block arrangement is the ratio of the reciprocals of the variances used in calculating the data in columns 1 and 2. These ratios are given in column 3. Finally the efficiency of comparisons between strain means is given in the last column by the standard errors of the means of ten plots in the progeny row trial and of six plots in the small bulks trial. These again are expressed as percentages of the grand mean.

The elimination of block differences resulted in large increases in accuracy in most characters. Not only does such a design improve the accuracy of comparison. It gives reliable guidance on which characters can be most profitably subjected to selection. As a group, seed cotton and lint characters were accurately estimated, differences of the order of 5 % being detected with confidence even with five plant plots replicated ten times. Yields and bolls per plant were much more variable, the larger plots six times replicated only providing satisfactory evidence of the existence of differences of the order of 12-15 %. The accuracy is of much the same order, however, as that obtained in properly replicated variety trials, and is therefore satisfactory, in that the small plots have not greatly increased the variability.

There are serious disadvantages inherent in progeny row breeding, especially for areas in which wide fluctuations in soil and climate occur. With the small amount of material available and the detailed examination to which it is subjected, it is impossible to carry out tests under a representative range of conditions. To improve the accuracy of his comparisons the breeder usually chooses uniform, fertile fields for his trials, and thereby exposes himself to the criticism that his strains have been bred and tested for suitability to a set of conditions which does not exist in practice. Plant habit, proneness to lodge under a heavy crop, resistance to certain pests and diseases and so on, can only be estimated in large plots. Such characters as ability to give a fair yield even under bad conditions, and satisfactory response to the range of soils and seasons likely to be encountered are particularly important in countries with uncertain climates and wide ranges of soil conditions, as for example South Africa and Queensland. To meet these problems breeders reduce progeny row work and bulk up their material as quickly as possible for extensive testing. The greatest successes of progeny row breeding in cotton have been attained in countries with comparatively uniform climates, such as

the West Indies and Egypt. In this connexion Mason (1938) has suggested that "secondary" selection, which may be defined as selection in the immediate progeny of single plants, has been considerably overemphasized at the expense of "primary" selection which includes single plant selection in unselected populations, hybrid material and improved bulks multiplied up from earlier selections. He pointed out that most of the outstanding successes in cotton breeding in India and Africa have been due to "lucky finds" and not to steady progress in orthodox breeding plots. One very important reason for this is that in new cotton-growing countries, or where a new and powerful factor is introduced into the situation, such as the invasion of a disease or pest, the breeder can sample a variable crop population with a reasonable expectation of making considerable progress in a short time. Also, until the development of the randomized progeny row technique, the testing of breeding material was so inefficient that the small differences on which steady improvement under selection should be based were only detected with difficulty. Under the circumstances secondary selection has been as successful as could be expected, as for example in the production of a steady improvement in lint and seed cotton characters under the comparatively uniform climatic conditions of Egypt and the West Indies.

IV. DISCUSSION

There can be no response to selection unless the material is genetically variable. The relation of variability to rate of change under selection, and the effect of selection in reducing variability are therefore fundamental factors in breeding theory. The examination of unselected crop populations has provided information on the equilibrium between selection and variability that is established in nature, and it appears that variability persists at a high level. It follows that natural selection does not necessarily result in uniformity, and the stability of such mixtures must be due to selection and not to genetic uniformity.

The records of breeding projects with Sea Island cotton show that it is in practice impossible to achieve genetic uniformity, even when it is deliberately sought. Variance may be greatly reduced, but it persists in some measure in even the most closely bred strains. If the stability of unselected populations is due to selection and not to genetic uniformity, the breeder may regard purity as a secondary consideration, and a new approach to his problem is possible.

The problems involved in the choice of material for selection have been better studied than most others that face the breeder. For most

crops the areas of high variability are known. Now that it is recognized that hybridization is only a means of increasing variability, and is a preliminary to selection and not a substitute for it, breeding programmes are better planned. No one nowadays wastes his time creating variability when it exists in his neighbour's fields. Where hybridization is necessary, genetic investigations have marked off the dangerous areas where cytological abnormalities and interspecific breakdown require special treatment, and in some cases, as in cotton, sound guidance can be given as to the type of variability and the order of its magnitude likely to be found in hybrids of any given type.

The chief importance of the application of modern statistical layouts to progeny row material lies in the demonstration that breeding material can be subjected to rigorous tests at any stage beyond the single plant. Early testing is obviously a great advantage for some characters. When, for example, certain minimum lint characters are required in a cotton strain, all progenies failing to reach the standard can be discarded immediately, enabling a more rigorous selection to be exercised for yield and flexibility of response when the remaining material has been bulked up. There is now no excuse for uncritical breeding work. It is possible to determine for each character the earliest stage at which efficient selection and critical comparison can be made, and a programme of progressive sifting and testing can be built up. The argument that flexibility is of prime importance is no excuse for inefficient testing.

In these problems considerable progress has already been made. There remain lines of enquiry more specifically genetic in the narrow sense on which work has hardly been started. The analysis of yield into its components was one of the early advances in cotton breeding. Its logical counterpart, studies of the synthesis of yield under different circumstances and in different strains, has hardly been touched. The development of discriminant functions (Fisher, 1936; Fairfield Smith, 1936) provides a tool with which the study can be undertaken. At present there is some knowledge of what characters can most easily be influenced by selection, but nothing is known of the extent to which a change in one character will result in compensating changes, which may perhaps be less easily detected, in others.

Studies of the rate and magnitude of change that can be induced by selection have an obvious bearing on breeding policy, but little information is available beyond "Student's" (1933) analysis of Winter's selection experiment, Harland's (1934) account of the breeding of Montserrat Sea Island cotton and Hutchinson & Kubersingh's (1936) analysis of the

282 *The Application of Genetics to Plant Breeding*

effect of selection on Malvi cotton. A preliminary enquiry into another aspect of the problem of the mass action of genes is Anderson's (1939) recent calculation of the limiting effect of linkage on gene assortment in distant hybrids. This has an interesting bearing on the rate of re-establishment of the species balance in Harland's backcrossing method of exploiting interspecies hybrids.

Enough has been said to indicate the great range of fundamental problems facing the plant breeder. Many of them fall outside the scope of genetics as the subject is now studied, and it is one of the attractions of the attempt to develop an applied branch of genetics that the stimulus of practical needs opens up lines of enquiry of wide theoretical interest.

REFERENCES

- ANDERSON, E. (1939). *Amer. Nat.* **73**, 185.
 BALLS, W. L. (1919). *The Cotton Plant in Egypt*. London: Macmillan.
 BROWN, C. H. (1932). *E.C.G. Rev.* **9**, 119.
 — (1939). *E.C.G. Rev.* **16**, 111.
 FISHER, R. A. (1936). *Ann. Eugen., Lond.*, **7**, 179.
 FISHER, R. A., IMMER, F. R. & TEDIN, O. (1932). *Genetics*, **17**, 107.
 HARLAND, S. C. (1920). *W. Ind. Bull.* **17**, 145, 210.
 — (1932). *Bibliogr. Genet.* **9**, 107.
 — (1934). *Rep. 2nd Conf. on Cotton Gr. Problems*, p. 31. E.C.G.C. London.
 — (1936). *Biol. Rev.* **11**, 83.
 HUTCHINSON, J. B. (1938 a). *Proc. 1st Conf. Sci. Res. Workers on Cotton in India*, p. 347. I.C.C.C. Bombay.
 HUTCHINSON, J. B. & KUBERSINGH (1936). *Ind. J. Agric. Sci.* **6**, 672.
 HUTCHINSON, J. B. & GHOSE, R. L. M. (1937 a). *Ind. J. Agric. Sci.* **7**, 1.
 — (1937 b). *Ind. J. Agric. Sci.* **7**, 233.
 HUTCHINSON, J. B. & PANSE, V. G. (1937 c). *Ind. J. Agric. Sci.* **7**, 531.
 HUTCHINSON, J. B., PANSE, V. G., APTE, N. S. & PUGH, B. M. (1938 b). *Ind. J. Agric. Sci.* **8**, 131.
 HUTCHINSON, J. B., PANSE, V. G. & GOVANDE, G. K. (1938 c). *Ind. J. Agric. Sci.* **8**, 757.
 KING, H. E. (1938). *Rep. 3rd Conf. on Cotton Gr. Problems*, p. 139. E.C.G.C. London.
 KNIGHT, R. L. & CLOUSTON, T. W. (1939). *J. Genet.* **38**, 133.
 MASON, T. G. (1938). *E.C.G. Rev.* **15**, 113.
 PATEL, L. P. (1920). *Mem. Dep. Agric. Ind. (Bot. Ser.)*, **11**, no. 4.
 PEAT, J. E. & PRENTICE, A. N. (1939). *Progress Reports from Exp. Stations (S. Rhodesia)*, E.C.G.C. London.
 SILOW, R. A. (1939). *J. Genet.* **38**, 229.
 SMITH, H. FAIRFIELD (1936). *Ann. Eugen., Lond.*, **7**, 240.
 STOCK, T. D. (1927). *Mem. Dept. Agric. Ind. (Bot. Ser.)*, **14**, 177.
 "STUDENT" (1933). *Eugen. Rev.* **24**, 293.
 TROUGHT, T. (1934). *Rep. 2nd Conf. on Cotton Gr. Problems*, p. 51. E.C.G.C. London.
 VAVILOV, N. I. (1935). "Botanical-Geographical Principles of Selection." *Leningrad Acad. Agr. Sci. of Inst. Pl. Br. in U.S.S.R.*

APPLICATION OF GENETICS TO PLANT BREEDING

II. THE INHERITANCE OF QUANTITATIVE CHARACTERS AND PLANT BREEDING¹

By V. G. PANSE

Galton Laboratory, University College, London

CONTENTS

	PAGE
I. Introduction	283
II. The analysis of experimental data	284
(a) Experimental material	284
(b) The regression of F_3 progenies on F_2 plants	285
(c) Estimation of genetic variance	287
(d) Effective number of factors	289
III. Models of genetic factors	290
IV. Discussion	295
V. Summary	298
References	299
Appendix	300

I. INTRODUCTION

It is pointed out in Part I of this contribution and is now generally agreed that the influence of genetics on plant breeding has not fulfilled the early expectations. While there is no doubt that the development of the subject has affected the general outlook of the breeder, the detailed guidance to which he has been looking forward is still lacking. For example, the breeder is now conscious that the selective capacity of his material depends upon the amount of heritable variability present, but it is still necessary to understand in what manner this variability functions, in order to be able to estimate it and use it explicitly in breeding. Similarly, an analysis of the genetic situation in the character under breeding is of vital importance, for it is on such points as the number and magnitude of the factors and their dominance and epistatic relations that the maximum improvement attainable, the rate of improvement by selection and also the most efficient procedure of selection depend. In studying these aspects of the genetical situation and the effect of selection, the influence of the environment cannot be neglected, since, as it is well established,

¹ Read at the Seventh International Genetic Congress, Edinburgh, August 1939.

all quantitative characters are susceptible to the environment and the variability from this source persists in a considerable measure in spite of all the refinement in the experimental technique. A study of the variance in F_2 progenies of crosses between three strains of cotton by Hutchinson *et al.* (1938) can be quoted as an example on this point.

Fisher *et al.* (1932) have pointed out the difficulties in the study of the genetics of quantitative characters and have given an outline of a statistical approach to the problem. They have shown that it is impossible to apply the usual method of genetic analysis here, and, therefore, new and essentially statistical methods must be developed. Not much progress, however, in handling actual experimental data on these lines appears to have been made so far. One of the few examples is "Student's" (1934) estimation of the minimum number of genes determining oil content in Winter's selection experiment. Recently, Charles & Smith (1939) have given statistical criteria for distinguishing between the arithmetic and geometric type of gene action in quantitative inheritance.

The results of a statistical study of the inheritance of staple-length in cotton having a bearing on some of the points mentioned above are reported here. Staple-length is the mean length of the cotton fibres and is the principal character governing spinning quality. The results are by no means complete and are chiefly intended to illustrate a possible mode of attack on the problem of quantitative inheritance. In presenting them the genetical and plant breeding aspects are emphasized and the mathematical details reduced to a minimum. The statistical method used is, however, of a general application and will be published separately.

II. THE ANALYSIS OF EXPERIMENTAL DATA

(a) *Experimental material*

The experimental data used in the present work were obtained from crosses between three strains of Indian cotton (*G. arboreum* var. *neglectum*), Bani, Malvi and C 520. The sources of these strains and their agricultural behaviour are described elsewhere (Hutchinson *et al.* 1938). All strains were self-fertilized and line-bred at Indore in Central India for eight generations before the crosses used in the experiments were made in 1934. F_1 's were grown in 1935. Taking four F_1 plants from each cross, the resulting F_2 progenies were grown in 1936 in a randomized progeny row trial (Hutchinson & Panse, 1937) with five blocks, each plot consisting of a single row of ten plants. The experiment suffered from defective germination and later from a rather severe attack of wilt, and consequently the number of plants at the time of harvest was only about

50 % of the expected. Ten plants from each F_2 progeny were selected at random, and the F_3 progenies from these were grown in 1937 in two blocks, each progeny-plot consisting of ten plants as before. Instead of randomizing all the 120 progenies together in each block, the twelve families, four from each cross and each family consisting of ten sister progenies arising from a common F_2 progeny, were first randomized and within each family-plot the sister progenies were randomized. The chief object of employing this experimental design was to reduce the environmental contribution to the variances by providing blocks, and further in the F_3 trial, by growing the sister progenies in close proximity of one another. Only self-fertilized seed was used for sowing and seed cotton from selfed flowers was picked for each plant separately and was generally used for examination. The staple-length to which the present data refer was measured on five random seeds per plant. The method of measurement is described elsewhere (Panse, Appendix III to Hutchinson & Ramiah, 1938).

(b) *The regression of F_3 progenies on F_2 plants*

The analysis of variance of the F_2 data is given below for each cross separately:

		Cross								
		C 520 × Bani			C 520 × Malvi			Malvi × Bani		
Due to	...	D.F.	S.S.	M.S.	D.F.	S.S.	M.S.	D.F.	S.S.	M.S.
Blocks		4	12.844	3.211	4	5.210	1.302	4	5.950	1.487
Progenies		3	0.556	0.185	3	3.213	1.071	3	3.201	1.067
Plot error		12	20.051	1.671	12	13.719	1.143	12	11.943	0.995
Within plots		71	214.085	0.919	101	330.622	0.595	88	212.571	0.526

The first three items were obtained by analysing plot mean values. The sums of squares for the last item were calculated from individual plant values within each plot and pooled over all plots. The corresponding mean squares were further divided by the harmonic means of plant numbers in all plots in order to make them comparable with the mean squares obtained from plot values. A comparison between the mean squares for plot error and "within plots" gives the variance ratios 1.82, 1.92 and 1.89 for the three crosses respectively. The last two are significant on the 5 % level, while the first is slightly lower than the value required for significance. This means that plot to plot differences affect staple-length in addition to differences between plants in the same plot. A similar result was obtained with large plots ($\frac{1}{100}$ acre each) in field scale varietal trials in Central India (Hutchinson & Panse, 1935). In the present case, however, part of the differences is genetic.

The effect of plot differences on staple length is important in relation to the method of selection of the best plants for propagation. The value of the selected plant will partly depend on the plot to which it belongs, and, therefore, the mean value of the plot must also be taken into account. This point was studied in calculating the regression of F_3 progenies on F_2 plants. The relevant portions of the analyses of variance of the F_3 progenies are shown below.

		Cross								
		C 520 × Bani			C 520 × Malvi			Malvi × Bani		
Due to	...	D.F.	S.S.	M.S.	D.F.	S.S.	M.S.	D.F.	S.S.	M.S.
Progenies		35	98.63	2.818	36	106.54	2.959	34	53.38	1.570
within families										
Plot error		35	21.96	0.628	36	27.71	0.770	34	11.65	0.343

Data of one progeny in the first cross and of two in the third were discarded on account of a doubt regarding their correctness. A portion of the sum of squares between progenies can be accounted for by the regression of the progeny mean on the value of the F_2 parent plant. In calculating this regression the staple-length of the F_2 plant was used as one independent variate and the mean staple-length of the plot to which it belonged as the other, and a partial regression equation was obtained in terms of these two variates. The numerical values of the partial regression coefficients were as follows.

Regression coefficient	C 520 × Bani	C 520 × Malvi	Malvi × Bani
b_1 (plant)	0.5117	0.4816	0.1551
b_2 (plot)	-0.5808	-0.1022	-0.0311

The regression equations can be expressed with a slight modification in a more suitable form. If x_1 is the plant value, x_2 the mean plot value and $z = x_1 - x_2$ then $x_1 = z + x_2$ and,

$$b_1x_1 + b_2x_2 = b_1(z + x_2) + b_2x_2 = b_1(x_1 - x_2) + (b_1 + b_2)x_2.$$

The variate in the first term of the latter expression is now the plant value minus the plot value. The variate in the second term is the plot value as before, but the coefficient of this term is the sum of the two partial regression coefficients. Using the method of solving regression equations given by Fisher (1936, § 29), the standard errors for the regression coefficients in the modified equation are $s\sqrt{c_{11}}$ for b_1 and $s\sqrt{(c_{11} + 2c_{12} + c_{22})}$ for $b_1 + b_2$, where s is the standard deviation obtained from the residual sum of squares between progenies and c_{11} , c_{12} and c_{22}

are the elements of the multiplying matrix. The numerical values of the new coefficients and their standard errors are given below:

Cross	b_1	S.E. of b_1	$b_1 + b_2$	S.E. of $b_1 + b_2$
C 520 \times Bani	0.5117	0.1186	-0.0691	0.1267
C 520 \times Malvi	0.4816	0.1072	0.3794	0.1680
Malvi \times Bani	0.1551	0.1421	0.1239	0.1565

In the first two crosses the coefficient b_1 is highly significant. The coefficient $b_1 + b_2$ is positive and significant in the second cross, while it has a very low negative value in the first. The fact that this latter coefficient has no significant negative value in any of the three crosses, while in one of them it is significantly positive, clearly shows that instead of selecting plants for propagation on the merit of their own values, it is profitable to select them on the basis of the excess of their individual values over the mean values of the plots to which they belong. The plots will not then depress the regression by their negative contribution, while there is a possibility of a positive contribution to the regression from this source through genetic sampling, as the second cross indicates. In the third cross the coefficient b_1 is non-significant and small. One explanation of this result would be that in this cross the phenotypic values of the F_2 plants chosen as progenitors were predominantly influenced by environment and were not, therefore, sufficiently correlated with their genetic values. Why the environmental effect should be so high in this particular cross is not clear.

(c) *Estimation of genetic variance*

The genetic portion of the F_2 variance can be estimated from the regression of F_3 progenies on F_2 parents. If x is the value of the parent plant and y the mean value of the resulting progeny, the regression coefficient of y on x is $\frac{Sy(x-\bar{x})}{S(x-\bar{x})^2}$. Now x can be considered as made up of a genetic component ξ and a non-genetic modification η , i.e. $x = \xi + \eta$. Then the expected value of y is ξ .

Therefore, the expected value of $y(x-\bar{x}) = E\{\xi(\xi-\bar{\xi}) + \xi(\eta-\bar{\eta})\}$. The term $E\xi(\eta-\bar{\eta}) = 0$, because ξ and η are independent of each other, while $E\xi(\xi-\bar{\xi})$ can be shown to be equal to $E(\xi-\bar{\xi})^2$, which is the expected genetic variance of the parent plants.

The expected value of $(x-\bar{x})^2 = E\{(\xi-\bar{\xi}) + (\eta-\bar{\eta})\}^2$, which is equal to $E(\xi-\bar{\xi})^2 + E(\eta-\bar{\eta})^2$. The first term represents the genetic and the second non-genetic variance of the parent plants. Therefore $\frac{Sy(x-\bar{x})}{S(x-\bar{x})^2}$, which is

an estimate of $\frac{E\{y(x-\bar{x})\}}{E(x-\bar{x})^2}$, is also an estimate of $\frac{\text{genetic variance}}{\text{total variance}}$ of the parent plants. When, as in the present case, the parent plants belong to different plots and the regression equation of the type, $y = b_1(\text{plant} - \text{plot}) + (b_1 + b_2)\text{plot}$, considered in the previous section is used, the value of the parent plant can be assumed to contain a genetic component, a non-genetic modification due to variation between plants in the same plot and a second similar modification arising from variation between plots. The expectation of the mean value of the progeny will again be equal to the genetic component of the parent and it can be shown (see Appendix) that the regression coefficients b_1 and $b_1 + b_2$ are expressible in terms of the expected genetic and non-genetic variances of the parents as follows:

$$b_1 = \frac{\text{genetic variance}}{\text{total variance within plots}}, \text{ and } b_1 + b_2 = \frac{\text{genetic variance}}{\text{total variance between plots}}$$

Applied to the present data, the numerical value of the regression coefficient b_1 represents the fraction of the total variance within F_2 plots which is genetic. These are given below. The cross Malvi \times Bani is omitted on account of the failure of the regression pointed out before:

Cross	Total variance within plots, per plant	b_1	Genetic variance of F_2	Non-genetic variance within F_2 plots
C 520 \times Bani	3.015	0.5117	1.543	1.472
C 520 \times Malvi	3.273	0.4816	1.576	1.697

Following Fisher (1930, p. 33), the term "genetic" is used here with a special significance and refers to the effects of a strictly additive action of the genes concerned. It is in this sense that the mean value of the progeny is equal to the genetic value of the parent. The genetic variance of the parents estimated on this basis is also the result of the additive action of the genes and is distinguished from "genotypic" variance which is the total variance due to the segregation of genes and includes, in addition to the genetic variance, variance arising from non-additive interactions of genes such as dominance and epistacy. The presence of these interactions tends to depress the regression of the progeny mean on the parental value by causing the genotypic variance of the parents to be greater than the genetic.

The non-genetic fraction of the F_2 variance shown above may thus include the difference between the genotypic and genetic variance in addition to the environmental variance. The fact that it is as high as half of the total variance is important, since selection of the desired genetic values is rendered more and more uncertain as the non-genetic com-

ponent of the variance increases in proportion to the genetic, and consequently the rate of progress by selection is lowered.

(d) *Effective number of factors*

The mean genotypic variance within F_3 progenies is half of the F_2 genotypic variance (Fisher *et al.* 1932), while the variance of the mean variance within F_3 can be shown to be equal to one-quarter of the sum of squares of the variances due to individual factors. If, for example, the variances due to two factors **A** and **B** are a and b respectively, the variance within F_3 progenies will be due to the segregation of the heterozygotes only, progenies of homozygous F_2 individuals having no variance. Now,

F_3 progenies heterozygous for	Frequency of the progenies	Variance within the progeny
A and B	$\frac{1}{4}$	$a + b$
A only	$\frac{1}{4}$	a
B only	$\frac{1}{4}$	b
Neither	$\frac{1}{4}$	0
Mean variance within F_3 progenies		$\frac{1}{2} (a + b)$

And the variance of this variance =

$$\frac{1}{4} (a + b)^2 + \frac{1}{4} a^2 + \frac{1}{4} b^2 - \left\{ \frac{1}{4} (a + b) + \frac{1}{4} a + \frac{1}{4} b \right\}^2 = \frac{1}{4} (a^2 + b^2).$$

If the two factors are linked a different result is obtained; because now,

F_3 progenies heterozygous for	Frequency of the progenies	Variance within the progeny
A and B	$\frac{1}{2} (p^2 + q^2)$	$a + b$
A only	pq	a
B only	pq	b
Neither	$\frac{1}{2} (p^2 + q^2)$	0

where p and q are the fractions of gametes in coupling and repulsion respectively. While the mean variance within F_3 is $\frac{1}{2} (a + b)$ as with unlinked factors, the variance of this variance is

$$\frac{1}{2} (p^2 + q^2) (a + b)^2 + pq a^2 + pq b^2 - \left\{ \frac{1}{2} (p^2 + q^2) (a + b) + pqa + pqb \right\}^2,$$

which can be shown equal to,

$$\frac{1}{4} (a^2 + b^2) + \frac{1}{2} (p - q)^2 ab.$$

With linkage, therefore, the variance of the F_3 variance is increased by a quantity $\frac{1}{2} (p - q)^2 ab$. Below are given the values of this increase as a fraction of ab for various cross-over fractions:

q	$\frac{1}{2} (p - q)^2$
0	0.5
0.1	0.32
0.2	0.18
0.3	0.08
0.4	0.02
0.5	0

With close linkage, i.e. up to 20 % crossing-over, the increase in variance is appreciable but is insignificant beyond this point. With close linkage, however, the two factors will behave in segregation as a single factor and can be effectively counted as one. Therefore, the increase in variance due to linkage need not be taken into consideration in estimating the number of independent factors concerned with a given character.

As shown above, if the F_2 variance is $(a+b+...)$, the mean variance within F_3 progenies is $\frac{1}{2}(a+b+...) = V_3$, and the variance of this variance is $\frac{1}{4}(a^2+b^2+...) = V(V_3)$. Assuming the segregation in F_2 to be due to n factors all with equal variance α , then, $V_3 = \frac{n\alpha}{2}$ and $V(V_3) = \frac{n\alpha^2}{4}$.

$$\therefore V_3^2/V(V_3) = \frac{n^2}{4} \alpha^2 / \frac{n}{4} \alpha^2 = n,$$

where n the number of factors, hypothetically with equal variance and without linkage, can be termed the "effective" number of factors. This number can thus be calculated if the mean genotypic variance within F_3 progenies and the variance of the mean variance within F_3 progenies is known. These quantities are shown below for the staple-length data assuming the genotypic variance to be the same as genetic.

Cross	V_3 ($=\frac{1}{2}$ genotypic variance in F_2)	$V(V_3)$	$V_3^2/V(V_3) = n$
C 520 \times Bani	0.772	0.363	1.64
C 520 \times Malvi	0.788	0.224	2.77

This calculation does not mean that the actual number of factors is necessarily only two or three in the above crosses. The effective number of factors n is merely a ratio of the square of the mean genotypic variance within F_3 to its variance and is, therefore, equivalent to any number of factors with different magnitudes that satisfy this ratio. Another condition governing such a series of factors is obviously a given F_2 genotypic variance. Using the C 520 \times Malvi cross to supply the data for these conditions, alternative possibilities in respect to the genetic constitution of the F_2 which satisfy them will be considered in the next section. For simplification of calculations the genotypic variance in F_2 will be taken as 1.5 and an environmental component of equal magnitude. The value of n will be equated to 3, the nearest whole number to the calculated ratio.

III. MODELS OF GENETIC FACTORS

In setting up hypothetical systems of factors for staple-length segregation in the C 520 \times Malvi cross, the two extreme cases considered are (1) three equal factors, i.e. the actual number of factors equal to the

effective number, and (2) a very large number of unequal factors amounting to an infinite series. Assuming the F_2 distribution symmetrical, this can be due either to the absence of dominance in the factors concerned or due to equal dominance in opposite directions, the two types of dominance balancing each other. The latter case deserves special notice for, as will be seen later, it really makes possible the consideration of consequences of a dominance bias in one direction and thus, while keeping the algebraic calculations much simpler, makes a separate examination of the cases with dominance in one direction only, unnecessary in principle. The infinite series of factors will be represented by a single convergent geometric series without dominance and by two such equal series with dominance in opposite directions.

In forming a geometric series of factors with F_2 variance equal to $\frac{3}{2}$ and $V_3^2/V(V_3)=3$, if $1/r$ is the common ratio between the variances of individual factors and a the variance of the first member of the series, it can be shown that $\frac{r+1}{r-1} = V_3^2/V(V_3)=3$, and the F_2 variance $= \frac{ar}{r-1} = \frac{3}{2}$. Therefore, $1/r = \frac{1}{2}$ and $a = \frac{3}{4}$. The factors without dominance giving these variances are, therefore, arranged in a geometric series beginning with $\sqrt{\frac{3}{2}}$ and having the common ratio $\sqrt{\frac{1}{2}}$. With two equal geometric series the F_2 variance due to each is $\frac{3}{4}$ and the ratio $\frac{r+1}{r-1} = \frac{3}{2}$, making the first variance in each series $\frac{3}{8}$ with a common ratio $\frac{1}{2}$. With complete dominance in opposite directions factors in each series, therefore, begin with a magnitude of $2/\sqrt{5}$ and have the common ratio $1/\sqrt{5}$.

The various systems of factors considered are,

I. No dominance, factors in a geometric series and represented as,

$$\left. \begin{array}{lll} AA + \sqrt{\frac{3}{2}} & BB + \sqrt{\frac{3}{4}} & CC + \sqrt{\frac{3}{8}} \\ Aa & 0 & Bb & 0 & Cc & 0 \\ aa - \sqrt{\frac{3}{2}} & bb - \sqrt{\frac{3}{4}} & cc - \sqrt{\frac{3}{8}} \end{array} \right\} \text{etc., with the common ratio } 1/r = 1/\sqrt{2}.$$

The highest possible genotypic value on this set up will be,

$$\frac{\sqrt{\frac{3}{2}}}{1 - 1/\sqrt{2}} = \frac{\sqrt{3}(\sqrt{2}+1)}{(\sqrt{2}-1)(\sqrt{2}+1)} = 4.182,$$

corresponding to the genotype **AABBCC**....

II. No dominance, three equal factors which of course have equal variances. The factors are

$$\begin{array}{lll} AA + 1 & BB + 1 & CC + 1 \\ Aa & 0 & Bb & 0 & Cc & 0 \\ aa - 1 & bb - 1 & cc - 1 \end{array}$$

the highest genotype **AABBCC** having the value 3.

III. Balanced dominance, factors in two equal geometric series with complete dominance in opposite directions. Each series will contribute $\frac{3}{4}$ to F_2 variance and have the ratio $n = \frac{3}{2}$. The two series can, therefore, be represented as follows.

$$\begin{array}{l} \text{Series I: } \left. \begin{array}{lll} \mathbf{AA} + 2/\sqrt{5} & \mathbf{BB} + \frac{2}{5} & \mathbf{CC} + 2/5\sqrt{5} \\ \mathbf{Aa} + 2/\sqrt{5} & \mathbf{Bb} + \frac{2}{5} & \mathbf{Cc} + 2/5\sqrt{5} \\ \mathbf{aa} - 2/\sqrt{5} & \mathbf{bb} - \frac{2}{5} & \mathbf{cc} - 2/5\sqrt{5} \end{array} \right\} \text{ etc.,} \\ \text{Series II: } \left. \begin{array}{lll} \mathbf{A'A'} + 2/\sqrt{5} & \mathbf{B'B'} + \frac{2}{5} & \mathbf{C'C'} + 2/5\sqrt{5} \\ \mathbf{A'a'} - 2/\sqrt{5} & \mathbf{B'b'} - \frac{2}{5} & \mathbf{C'c'} - 2/5\sqrt{5} \\ \mathbf{a'a'} - 2/\sqrt{5} & \mathbf{b'b'} - \frac{2}{5} & \mathbf{c'c'} - 2/5\sqrt{5} \end{array} \right\} \text{ etc.,} \end{array}$$

each with common ratio $1/r = 1/\sqrt{5}$. The highest genotype

$$\mathbf{AAA'A'BBB'B'CCC'C' \dots}$$

will be scored as,

$$2 \times \frac{2/\sqrt{5}}{1 - 1/\sqrt{5}} = \sqrt{5} + 1 = 3.236.$$

IV. Balanced dominance, three factors with equal variance, one of them without dominance and the other two with complete dominance in opposite directions. They are,

$$\begin{array}{lll} \mathbf{AA} + \sqrt{\frac{2}{3}} & \mathbf{BB} + 1 & \mathbf{CC} + \sqrt{\frac{2}{3}} \\ \mathbf{Aa} + \sqrt{\frac{2}{3}} & \mathbf{Bb} & 0 & \mathbf{Cc} - \sqrt{\frac{2}{3}} \\ \mathbf{aa} - \sqrt{\frac{2}{3}} & \mathbf{bb} - 1 & \mathbf{cc} - \sqrt{\frac{2}{3}} \end{array}$$

The highest genotype **AABBCC** has the value 2.633.

V. Balanced dominance, three factors of equal magnitude with dominance as in the last case. These are,

$$\begin{array}{lll} \mathbf{AA} + \sqrt{\frac{3}{4}} & \mathbf{BB} + \sqrt{\frac{3}{4}} & \mathbf{CC} + \sqrt{\frac{3}{4}} \\ \mathbf{Aa} + \sqrt{\frac{3}{4}} & \mathbf{Bb} & 0 & \mathbf{Cc} - \sqrt{\frac{3}{4}} \\ \mathbf{aa} - \sqrt{\frac{3}{4}} & \mathbf{bb} - \sqrt{\frac{3}{4}} & \mathbf{cc} - \sqrt{\frac{3}{4}} \end{array}$$

This case which is a variation of case IV is also comparable to case II on account of the equal size of the three factors; but it should be noted that the ratio n is here $32/11$ which is very slightly less than the required value. The highest genotypic value is 2.598.

The statistical consequences in the F_3 generation of selecting as progenitors a certain proportion of individuals in a given range of the F_2 populations with these hypothetical genetic constitutions can be studied with the help of a moment generating function. The F_3 properties determined are (1) the mean value of the progenies, (2) the mean genotypic

variance within the progenies, (3) the covariance of means and variances of the progenies, (4) the variance of the mean values of the progenies, and (5) the variance of the mean variance within the progenies. A selection of 10 % of the F_2 individuals with the highest phenotypic values was assumed for this purpose.

It is not intended to enter into any detail of the calculation here, but the method is briefly indicated. For each of the genetic models an exact simultaneous distribution of the F_2 phenotypic values, the mean values of the resulting progenies and the genotypic variance within these progenies can be set down in the form of a moment-generating function.

TABLE I

Highest genotypic values and the statistical properties of F_3 from selected F_2 's with different genetic constitutions

System of factors	Highest genotypic value	Genotypic mean of F_3 progenies	Mean genotypic variance within F_3 progenies	Variance of the genotypic mean of F_3 progenies	Covariance between F_3 means and variances	Variance of the mean genotypic variance within F_3 progenies
I. No dominance, factors in a geometric series	4.182	1.512	0.595	0.744	-0.182	0.160
II. No dominance, three equal factors	3.000	1.512	0.598	0.749	-0.196	0.158
III. Balanced dominance, factors in two geometric series	3.236	1.266	0.642	0.669	-0.125	0.146
IV. Balanced dominance, three factors with equal variance	2.633	1.347	0.629	0.693	-0.155	0.153
V. Balanced dominance, three equal factors	2.598	1.328	0.632	0.697	-0.146	0.153

By expanding it, it is possible to obtain the various sums of powers and of products necessary to express the F_3 properties or quantities related to them in terms of the F_2 phenotypic values by means of a regression equation of the type, $Y = A + Bx + Cx^2 + Dx^3 + Ex^4 \dots$, where Y represents the particular F_3 quantity and x the F_2 phenotypic value. An equation of the fourth degree was found satisfactory. Since Y is expressed in terms of x whose exact distribution is known, the mean value of Y over a particular range of this distribution can be determined by integrating between the limits appropriate to the degree of selection applied. The assumption of a symmetrical F_2 distribution has made the algebraic work much easier but such an assumption is not necessary to use the method. The results obtained are given in Table I.

These theoretical or population values can be used for two purposes. The last five quantities can be easily calculated from experimental

data on F_2 and F_3 progenies, the latter grown from 10 % of the F_2 individuals selected for highest phenotypic values. By a comparison of the two sets of values it should be possible to discover the presence or absence of dominance in the experimental material, and whether the observed segregation is due to a few large factors or a large number of factors of varying magnitudes. Secondly, the values set out in the table can be used to compare the effects on F_3 of applying selection to F_2 populations of different genetic constitutions and to obtain some indication of the course of further selection.

For comparing experimental with theoretical values, the standard error can be used for the mean value of the F_3 progenies and a χ^2 test for the three variances, since the sum of squares obtained from n sample values divided by the population variance is a χ^2 with $n-1$ degrees of freedom. The covariance cannot be compared directly because it is dependent on the variance of mean values and of variances of F_3 progenies. Where both these variances agree with their theoretical values, the covariance can be tested as a correlation coefficient and where only one of them agrees as a regression coefficient. A separate test of covariance is of no use when both variances differ significantly from their population values. In the present case a comparison of the three variances with their experimental estimates is not likely to be helpful as in these quantities the five models differ very little from one another. The mean value of F_3 progenies and the covariance on the other hand show somewhat wider differences and appear more useful. The experimental data to make the comparisons are not available.

The highest genotypic values given in the table show the limits to which selection can be carried in each case. For equivalent number of factors these limits are higher in the absence of dominance than in its presence, and within each type higher when a given genotypic variance is caused by a large number of factors with varying magnitudes than when it is due to a few large factors. The genotypic mean values of the F_3 progenies show the advance resulting from selection as the mean value of the whole F_3 is zero in each case. A greater progress has been made in the absence of dominance than in its presence, and in the latter case three factors have raised the mean value higher than an infinite series. Considered in relation to the highest values attainable, the progress made in F_3 with three factors with and without dominance represents 51 and 50 % of the maximum respectively, and 39 and 36 % with an infinite series of factors. The ratio V_3^2/V (V_3) calculated from the table indicates on the same scale as in the whole F_3 population, the "effective" number of

factors operating in the selected portion of the population. Its calculated values, which are given below, show the greatest reduction in heterozygosity when dominance is absent, and in its presence a greater reduction with three factors than with an infinite number.

System of factors	$V^2_3/V (V_3)$
I. No dominance, geometric series	2.210
II. No dominance, three equal factors	2.265
III. Balanced dominance, geometric series	2.335
IV. Balanced dominance, three factors with equal variances	2.586
V. Balanced dominance, three equal factors	2.611

The genotypic variance within the F_3 progenies is now approximately of the same magnitude as that between progenies, whereas in the whole F_3 the latter is twice as great as the former in the absence of dominance and one and a half times as great in its presence (Fisher *et al.* 1932). Even so, further selection based on progeny means will be more efficient than selecting individual values, because the latter will be affected by environmental variation to a considerably greater extent. The negative covariance between progeny means and variances in all the five cases indicates on average a falling off of the genotypic variance of a progeny as its mean value increases. To achieve its object selection of progenies with high mean values is necessary, but this at the same time enforces selection of individuals in progenies with a relatively low genotypic variance, and a larger number of individuals must, therefore, be selected to ensure high genotypic values. The weight to be attached to the progeny mean and the individual plants selected for further propagation must be studied and a balance maintained between the two.

The present analysis must be extended to F_4 and subsequent generations to study the rate at which the different properties of the populations with different genetic constitutions are modified under selection. It is also possible that the various statistical quantities calculated for F_3 will show a wider distinction between the different genetic systems at some other level of selection in F_2 , than the one adopted.

IV. DISCUSSION

In Part I, Hutchinson has discussed the superiority of progeny row breeding to mass selection and the advantage of selecting on the basis of the progeny means. When selection is to be continued in the progeny, the usual practice is to select a certain number of individuals with the desired phenotypic values and grow further progenies from them. On account of the amount of material involved it is usually necessary to restrict the number of individuals whose progenies can be carried forward, and hence

it becomes all the more important that the individuals possess a high genetic potentiality. The result obtained with the regression of F_3 progenies on F_2 plants shows that when a progeny is grown in replicated plots, selection of individuals in the progeny should be based on the excess of the individual value over the mean value of the plot to which it belongs, as by so doing, the environmental influence on individual values of differences between plots can be eliminated.

In the present study the different Mendelian factors are supposed to act independently and the complicating feature of interaction between factors has been excluded. The phenomenon of dominance or the interaction between the phases of the same factor is, however, taken into account. Fisher (1918) has pointed out that the statistical effects of dominance and epistacy are similar. He has shown that the hypothesis of cumulative Mendelian factors fits the inheritance of human stature very accurately and it is important to examine how far it can explain the observed facts in plant breeding. The effects of dominance on selection have been clearly brought out in the present results. Before discussing them it should be noted in what manner dominance plays its part in the genetic models with balanced dominance. In the F_2 distribution heterozygotes with dominance for low values will accumulate in the lower portion and those with dominance for high values in the upper part. Selection for high phenotypic values will, therefore, almost entirely be restricted to phenotypes with dominance for high values and this dominance will show its effects in the F_3 . Thus the assumption of a symmetrical F_2 distribution has not prevented the consideration of dominance.

The table of F_3 values shows the retarding effect of dominance on selection. Comparing the two groups of cases with and without dominance, it will be seen that while starting with the same amount of genotypic variance in the F_2 , selection has raised the mean value of the F_3 higher in the absence of dominance than in its presence. In the former case, however, the whole of the F_2 genotypic variance is genetic while in the latter the genetic variance is lower, a part of the genotypic variance being due to dominance. This demonstrates how it is the genetic portion of the variance that determines the immediate capacity of the material for selection. The lower value of the covariance between means and variances of F_3 progenies in the presence of dominance also suggests that a part of the genotypic variance is not associated with mean values. Dominance also counteracts the other effect of selection viz. increase of homozygosity. It will be seen that a greater amount of genotypic variance persists in the F_3 when dominance is present and the ratio

$V_3^2/V(V_3)$ is similarly higher. This ratio, it will be remembered, corresponds to the effective number of factors. Dominance thus appears to slow down the change both in mean values and variances brought about by selection.

The estimate of genetic variance made by the regression of F_3 progenies on F_2 plants was also used as the genotypic variance in studying the different genetic systems. This was necessary in the absence of suitable experimental data for estimation of the latter. Actually, as pointed out before, the genotypic variance will be somewhat greater than genetic in the presence of dominance and has, therefore, been underestimated. In the three cases with balanced dominance it can be shown that the genetic variance of the F_2 is 22-33 % lower than the genotypic. The discrepancy between the two will become smaller if dominance is partial instead of complete as assumed here. For an accurate estimation of genotypic variation in F_2 it is necessary to grow progenies resulting from crossing F_2 plants among themselves at random. Then the F_2 genotypic variance is equal to twice the difference between the covariance of F_2 parental value with the mean of the F_3 offspring and the covariance of the F_2 parental value with the mean of its biparental offspring (Fisher *et al.* 1932).

On the question of the estimation of genotypic variance the paper by Charles & Smith, referred to in the Introduction, is of some interest. In developing criteria for distinguishing between the arithmetic and geometric types of gene action they are led to estimate the genotypic variance by taking the difference between the total and the environmental variance. They calculate the latter on the assumption that it is correlated with mean values in the three non-segregating generations viz. the two parents and the F_1 , and the same correlation holds in the segregating generations. In the absence of sufficient experimental evidence to justify this assumption their tests involving variances and skewness are of doubtful value. Under normal field-experimental conditions mean values and variances do not appear to show any such systematic relationship and the two are, therefore, commonly treated as independent. In the staple-length data of the cotton crosses no consistent relationship was observed between them in the parental and F_1 generations tested simultaneously in replicated trials for two years.

The hypothesis of geometrical effects used by these authors is that a given gene substitution multiplies the phenotypic value by a constant quantity characteristic of each factor. On this hypothesis they show that the mean value of the F_1 should be equal to the geometric mean of the two

parental values. As the geometric mean is always smaller than the corresponding arithmetic mean, this hypothesis will not be applicable to the large number of characters in which the F_1 has a value equal to or generally greater than the arithmetic mean of the two parents. Their tests involving mean values for the arithmetic effects of genes are capable of a general application with any type and magnitude of dominance. On the arithmetic hypothesis the mean value of the F_2 should be equal to the mean of the F_1 and the parental values, i.e. $F_2 = \frac{P_1 + P_2 + 2F_1}{4}$. Similarly, the two back cross means should each be equal to the mean of the F_1 and the parent involved. Using these tests on the staple-length data in the three crosses with F_2 values for two years and backcross values for one year, the agreement between the actual and expected values is found very close, the mean difference between the two being, 0.087 ± 0.175 and 0.173 ± 0.202 , respectively.

V. SUMMARY

The importance of the study of quantitative inheritance for a closer application of genetics to plant breeding has been recognized. The object of the present paper is to summarize the results obtained in a statistical study of quantitative inheritance relating to F_2 and F_3 progenies. The genetic and plant breeding aspects of the results are emphasized.

The experimental data used refer to the staple-length measurements on F_2 and F_3 progenies of crosses between strains of cotton belonging to the species *G. arboreum* var. *neglectum*, grown at the Institute of Plant Industry, Indore, Central India. The regression of mean staple-length of F_3 progenies on F_2 phenotypic values shows that it is advantageous to consider plot values and select individuals on the basis of their excess over the former where, as in the present case, inter-plot variation affects the character in addition to intra-plot variation. The coefficient of regression also gives an estimate of the genetic fraction of the total F_2 variance. This is an important relationship, as it affords a basis for separating the inheritable and non-inheritable components of variance in the experimental material.

The ratio of the square of the genotypic variance within F_3 progenies to the variance of this variance is shown to represent the "effective" number of factors which can account for the segregation in F_2 and which hypothetically possess equal variance and are without linkage. With a given F_2 variance and a given effective number of factors, it is possible to set up different genetic systems or models consisting of factors varying

in magnitude and number and with or without dominance. Five models with the smallest possible number of factors or an infinite number and each with or without dominance are considered.

By the use of a moment-generating function in three variables the moments of the distribution of the F_2 phenotype and of certain related quantities are calculated for each system. With these it is possible to express in terms of the F_2 phenotype properties of the F_3 progenies such as the genotypic mean or variance, and further to calculate their mean values in a portion of the F_3 population resulting from a selected proportion of F_2 phenotypes. Similar mean values obtained from experimental data can be compared with these theoretical values in order to discover the presence or absence of dominance and to decide on the possible number of factors operating in the experimental material.

In the present case, assuming a 10 % selection in F_2 , theoretical mean values for (1) the genotypic mean and (2) variance of F_3 progenies, (3) the covariance between the two, and (4) the variance of F_3 means and (5) of F_3 variances are calculated for each model. Their usefulness for identifying the genetic situation and the information obtained from them on the effect of selection applied and on questions relating to further selection are discussed.

The results summarized in the present paper form part of the work being carried out under the guidance of Prof. Fisher to whom I am indebted for much help. I should also like to thank my colleague Mr D. J. Finney at the Galton Laboratory for his help in discussing with me several mathematical points arising in the course of the work.

REFERENCES

- CHARLES, D. R. & SMITH, H. H. (1939). *Genetics*, **24**, 34.
FISHER, R. A. (1918). *Trans. Roy. Soc. Edinb.* **52**, 399.
— (1930). *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.
— (1936). *Statistical Methods for Research Workers*, 6th ed. London: Oliver and Boyd.
FISHER, R. A., IMMER, F. R. & TEDIN, OLOF (1932). *Genetics*, **17**, 107.
HUTCHINSON, J. B. & PANSE, V. G. (1935). *Ind. J. agric. Sci.* **5**, 545.
— (1937). *Ind. J. agric. Sci.* **7**, 531.
HUTCHINSON, J. B., PANSE, V. G. & GOVANDE, G. K. (1938). *Ind. J. agric. Sci.* **8**, 757.
PANSE, V. G. (1938). Appendix III to Hutchinson, J. B. and Ramiah, K., *Ind. J. agric. Sci.* **8**, 582.
"STUDENT" (1934). *Ann. Eugen., Lond.*, **6**, 77.

APPENDIX

To obtain the values of the partial regression coefficients b_1 and b_2 , it is necessary to solve the following simultaneous equations,

$$b_1 Sx_1^2 + b_2 Sx_1x_2 = Sx_1y, \quad \dots\dots (I)$$

$$b_1 Sx_1x_2 + b_2 Sx_2^2 = Sx_2y, \quad \dots\dots (II)$$

where y is the mean value of the progeny, x_1 the value of the parent plant and x_2 the mean value of the plot to which it belongs.

The expectations of sums of squares and of products in these equations are considered below.

Let there be n plants in each of m plots. If \bar{x} is the mean value of all mn plants, the value of any plant (x_1) can be scored as $\bar{x} + g + a + b$ and the mean value of a plot (x_2) as $\bar{x} + \frac{1}{n}(Sg + Sa + nb)$, g representing the genetic and a and b the environmental modifications due to variation within and between plots respectively. The corresponding expected variances can be set down as,

$$V_g = \frac{1}{mn} SSg^2, \quad V_a = \frac{1}{mn} SSa^2, \quad V_b = \frac{1}{mn} Snb^2 = \frac{1}{m} Sb^2.$$

These being independent of one another, the total variance between plants is $V_g + V_a + V_b$.

The covariance between plants and plot mean values

$$\begin{aligned} &= \frac{1}{mn} SS \{(x_1 - \bar{x})(x_2 - \bar{x})\} \\ &= \frac{1}{mn^2} SS \{(g + a + b)(Sg + Sa + nb)\} \\ &= \frac{1}{mn^2} S \{(Sg + Sa + nb)(Sg + Sa + nb)\} \\ &= \frac{1}{mn^2} (SSg^2 + SSa^2 + n^2 Sb^2) \\ &= \frac{1}{mn^2} (mn V_g + mn V_a + mn^2 V_b) \\ &= \frac{1}{n} (V_g + V_a) + V_b. \end{aligned}$$

The variance between plot mean values

$$\begin{aligned}
 &= \frac{1}{mn} \cdot nS (x_2 - \bar{x})^2 \\
 &= \frac{1}{m} S \left\{ \frac{1}{n} (Sg + Sa + nb) \right\}^2 \\
 &= \frac{1}{mn^2} (SSg^2 + SSa^2 + n^2 Sb^2) \\
 &= \frac{1}{n} (V_g + V_a) + V_b.
 \end{aligned}$$

The covariance between parent plants and progeny means

$$\begin{aligned}
 &= \frac{1}{mn} SS \{(x_1 - \bar{x}) (y - \bar{y})\} \\
 &= \frac{1}{mn} SS \{(g + a + b) g\},
 \end{aligned}$$

since the mean value of the progeny is equal to the genetic component of the parent plant,

$$\begin{aligned}
 &= \frac{1}{mn} SSg^2 \\
 &= V_g.
 \end{aligned}$$

The covariance between plot and progeny means

$$\begin{aligned}
 &= \frac{1}{mn} SS \{(x_2 - \bar{x}) (y - \bar{y})\} \\
 &= \frac{1}{mn^2} SS \{(Sg + Sa + nb) g\} \\
 &= \frac{1}{mn^2} SSg^2 \\
 &= \frac{1}{n} V_g.
 \end{aligned}$$

Substituting these expectations in equations (I) and (II) we get,

$$(V_g + V_a + V_b) b_1 + \left\{ \frac{1}{n} (V_g + V_a) + V_b \right\} b_2 = V_g, \quad \dots\dots\text{(III)}$$

$$\left\{ \frac{1}{n} (V_g + V_a) + V_b \right\} b_1 + \left\{ \frac{1}{n} (V_g + V_a) + V_b \right\} b_2 = \frac{1}{n} V_g. \quad \dots\text{(IV)}$$

Subtracting (IV) from (III) and multiplying throughout by n

$$\begin{aligned}
 (n V_g + n V_a - V_g - V_a) b_1 &= n V_g - V_g. \\
 \therefore (V_g + V_a) (n - 1) b_1 &= V_g (n - 1)
 \end{aligned}$$

and
$$b_1 = \frac{V_g}{V_g + V_a} = \frac{\text{genetic variance}}{\text{total variance within plots}}.$$

Substituting for b_1 in equation (III)

$$\left\{ \frac{1}{n} (V_g + V_a) + V_b \right\} b_2 = V_g - V_g \frac{(V_g + V_a + V_b)}{V_g + V_a}$$

$$= - \frac{V_g V_b}{V_g + V_a},$$

$$\therefore b_2 = - \frac{V_g V_b}{(V_g + V_a) \left\{ \frac{1}{n} (V_g + V_a) + V_b \right\}},$$

and

$$b_1 + b_2 = \frac{V_g}{V_g + V_a} - \frac{V_g V_b}{(V_g + V_a) \left\{ \frac{1}{n} (V_g + V_a) + V_b \right\}}$$

$$= \frac{V_g (V_g + V_a + n V_b) - n V_g V_b}{(V_g + V_a) (V_g + V_a + n V_b)}$$

$$= \frac{V_g (V_g + V_a)}{(V_g + V_a) (V_g + V_a + n V_b)} = \frac{V_g}{(V_g + V_a + n V_b)}$$

$$= \frac{\text{genetic variance}}{\text{total variance between plots}}.$$

THE ORIGIN AND EVOLUTION OF MULTIPLE SEX-CHROMOSOME MECHANISMS

By M. J. D. WHITE
University College, London

(With Six Text-figures)

CONTENTS

	PAGE
I. Introduction	303
(1) The nature of sex chromosomes	303
(2) The principles of chromosomal evolution	305
(3) Behaviour of sex chromosomes at meiosis	310
(4) The methods of investigation	311
II. Multiple sex chromosomes in Orthoptera (sens. lat.)	312
(1) Evolution of sex chromosomes in the <i>Acrididae</i>	312
(2) The case of <i>Paratylotropidia</i>	314
(3) Multiple sex chromosomes in mantids	317
(4) Multiple sex chromosomes in earwigs	319
III. Multiple sex chromosomes in the <i>Rhynchota</i>	319
(1) Sex chromosomes in Reduviids and in some other Heteroptera	319
(2) Sex chromosomes in <i>Cimex</i>	322
(3) Multiple sex chromosomes in Aphids	323
IV. The case of <i>Ascalaphus libelluloides</i>	323
V. The case of <i>Perla marginata</i>	324
VI. The case of <i>Phragmatobia fuliginosa</i>	324
VII. Multiple sex chromosomes in the Coleoptera	325
VIII. Sex chromosomes in the genus <i>Drosophila</i>	325
(1) The case of <i>Drosophila miranda</i>	325
(2) Rod-shaped and V-shaped X-chromosomes	329
IX. Multiple sex-chromosomes in the Arachnida	331
X. Multiple sex chromosomes in the Nematoda	332
XI. Summary	332
Addendum	333
References	334

I. INTRODUCTION

(1) *The nature of sex chromosomes*

IN the majority of bisexual species of animals the sex-chromosome mechanism depends on a single pair of chromosomes; that is to say, one sex possesses a pair of X-chromosomes, the other sex being either XY or XO (the "O" indicating merely the absence of a chromosome). The heterogametic (XY or XO) sex is usually the male, but in the Lepidoptera,

Trichoptera and birds it is the females which are heterogametic, the males being homogametic.

In all cases the *X*- and *Y*-chromosomes can be looked upon as a rather highly modified pair of autosomes whose sex-determining function depends on the fact that the *X* contains a special region (or regions) which is not present in the *Y* and which bears a number of sex-determining genes (female-determining in most groups, male-determining in the four mentioned above). The *Y* may also contain a special region or regions not found in the *X*. These differential regions may be very small or of considerable length; in fact, the evolutionary history of the *X*- and *Y*-chromosomes may be regarded as a progressive increase in size of the differential regions at the expense of the homologous ones, so that the *X* and *Y*, originally alike, come to be more and more unlike. They can never, however, become completely unlike; so long as a *Y*-chromosome remains at all it must contain a segment, however small, which can pair with the *X* at meiosis. (Certain animals in which there is no true pairing of the *X* and *Y* may be exceptions to this rule, and will be considered later.)

The earlier work on the genetics of *Drosophila* suggested that in the species of this genus the *Y*-chromosome may be more or less completely inert; later work has, however, shown that in *D. melanogaster* it does contain a number of genes which are necessary for fertility in the male. That the *Y* is essentially inert in many animals is suggested by the large number of groups in which it has become lost from the chromosome set altogether, the diploid number in the heterogametic set being an uneven one.

Cytological work has shown that, in *Drosophila* spp. at any rate, genetically inert regions may be recognized under the microscope because they are *heteropycnotic* at certain stages of mitosis and meiosis—that is to say, they thicken to a greater or less extent than the active chromosomal regions. If heteropycnosis can be regarded as a universal sign of genetical inertness (as appears probable), we are in the fortunate position of being able to predict the inertness of particular chromosomal regions in species of animals which have not yet been worked on genetically. In many animals the *X* as well as the *Y* is largely heteropycnotic, and in these cases it seems likely that the active sex-determining regions of the *X* are relatively small, most of the chromosome being, like the *Y*, inert.

The evolution of the sex-chromosome mechanism has been discussed by a number of authors, notably by Wilson (1925) and Darlington (1937, 1939). The present article will deal only with one particular aspect of the

problem, namely, the origin and subsequent fate of what may be called "multiple sex-chromosome mechanisms". Such a mechanism, as here defined, is a system of sex chromosomes involving more than one kind of X or one kind of Y . Where the X is represented by several chromosomes instead of by a single one they may be called X_1, X_2, X_3, \dots ; similarly, multiple Y 's can be called Y_1, Y_2, Y_3, \dots . A glance at Table I will show that these cases of multiple sex chromosomes occur rather sporadically in the Nematoda, Arachnida, Insecta and possibly in the Platyhelminthes. No case has been found as yet in the Vertebrata, since Painter's (1921) conclusion that certain lizards have multiple X -chromosomes must now be regarded, in the light of more recent work (Matthey, 1931), as erroneous. The whole subject of sex chromosomes was reviewed by Schrader (1928) in a very useful monograph; but some of the most interesting cases of "multiple" systems have been discovered since the publication of his book.

(2) *The principles of chromosomal evolution*

To the earlier cytologists these multiple sex-chromosome mechanisms appeared to be a relatively simple problem; they supposed that when an X_1 and an X_2 were present they simply represented the portions of an X -chromosome which had broken into two. Similarly, compound Y 's were looked upon as fragments of an originally single body.

Recent work on chromosome structure and behaviour has shown that this interpretation is probably in all cases untrue. In order to function properly at mitosis a chromosome must contain a special region called the spindle attachment (centromere or kinetochore). Chromosomes lacking this region, or possessing more than one spindle attachment, cannot become part of the permanent chromosome set of a species (Nawashin, 1932; Mather & Stone, 1933; Muller, 1940; but see White (1936) for an exceptional case of chromosomes with many spindle attachments). Mere fragmentation of a chromosome, such as the earlier workers envisaged, will consequently give rise to two portions, only one of which possesses a spindle attachment so that the other cannot persist through a number of cell divisions.

The general laws of chromosomal evolution are by now fairly well understood and have recently been summarized by Muller (1940). Broadly speaking, we may distinguish three main categories of events which can alter the chromosome set of a species: (1) gene mutation, (2) sectional rearrangements of parts of chromosomes, (3) loss or reduplication of whole chromosomes. With gene mutations, important though they

TABLE I

The known cases of multiple sex-chromosome mechanisms in animals

Group and species	Sex chromosomes in heterogametic sex	Author
Platyhelminthes		
<i>Schistosomum</i> (Bilharzia) <i>haematobium</i>	X_1X_2	Lindner, 1914
Nematoda		
<i>Ascaris incurva</i> (<i>Contracaecum incurvum</i>)	$X_1X_2X_3X_4X_5X_6X_7X_8Y$	Goodrich, 1914, 1916
<i>Ascaris lumbricoides</i>	$X_1X_2X_3X_4X_5$	Walton, 1924
<i>Belascaris triquetra</i>	X_1X_2	" "
<i>Ganguleterakis spinosa</i>	X_1X_2	" "
<i>Toxascaris canis</i>	$X_1X_2X_3X_4X_5X_6$	" "
Arachnida		
<i>Agalena naevia</i>	X_1X_2	Painter, 1914
<i>Amaurobius sylvestris</i>	X_1X_2	" "
<i>Anyphaena saltibunda</i>	X_1X_2	" "
<i>Callipes imbecilla</i>	X_1X_2	" "
<i>Dolomedes fontanus</i>	X_1X_2	" "
<i>Dugesiiella hentzi</i>	X_1X_2	" "
<i>Epeira scolopetaria</i>	X_1X_2	" "
<i>E. sericata</i>	X_1X_2	" "
<i>Lycosa communis</i>	X_1X_2	" "
<i>Maevia vittata</i>	X_1X_2	" "
<i>Oxyopes salticus</i>	X_1X_2	" "
<i>Schizocosa crassipes</i>	X_1X_2	Hard, 1939
<i>Xysticus triguttatus</i>	X_1X_2	Painter, 1914
Insecta		
Thysanura		
<i>Lepisma</i> (<i>Thermobia</i>) <i>domestica</i>	X_1X_2	Perrot, 1933
Plecoptera		
<i>Perla marginata</i>	X_1X_2	Junker, 1923
Saltatoria		
<i>Paratylotropidia brunneri</i>	X_1X_2Y	King & Beams, 1938
Mantoidea		
<i>Mantis religiosa</i>	X_1X_2Y	{ Oguma, 1921 King, 1931 Asana, 1934 White, unpublished
<i>Tenodera</i> spp.		
<i>Paratenodera sinensis</i>		
<i>Stagmomantis carolina</i>		
<i>Hierodula</i> sp.		
<i>Sphodromantis viridis</i>		
Dermaptera		
<i>Anisolabis</i> 3 spp.	X_1X_2Y	Morgan, 1928, Sugiyama, 1933
<i>Forficula auricularia</i> (some individuals)	X_1X_2Y	" "
Heteroptera		
Lygaeidae		
<i>Lygaeus equestris</i> (some individuals)	$XY Y$	von Pfaler, 1937
<i>Eremocoris erraticus</i>	X_1X_2Y	
Coreidae		
<i>Syromastes marginatus</i>	X_1X_2	Wilson, 1909 a, b
Pentatomidae		
<i>Thyanta calceata</i>	X_1X_2Y	Wilson, 1909 a, b

TABLE I (cont.)

The known cases of multiple sex-chromosome mechanisms in animals

Group and species	Sex chromosomes in heterogametic sex	Author
Galgulidae		
<i>Galgulus (Gelastocoris) oculatus</i>	$X_1X_2X_3X_4Y$	Payne, 1909, 1912a
Nepidae		
<i>Nepa cinerea</i>	$X_1X_2X_3X_4Y$	Steopoe, 1925
Reduviidae		
<i>Acholla multispinosa</i>	$X_1X_2X_3X_4X_5Y$	Payne, 1909, 1910
<i>Conorhinus sanguisugus</i>	X_1X_2Y	Payne, 1909, 1912a
<i>Diplocodus exsanguis</i>	X_1X_2Y	Payne, 1909
<i>Fitchia spinulosa</i>	X_1X_2Y	" "
<i>Pnirontis modesta</i>	$X_1X_2X_3X_4Y$	Payne, 1912a
<i>Prionidius cristatus</i>	$X_1X_2X_3Y$	Payne, 1909; Montgomery, 1901, 1906
<i>Pseliodes cinctus</i>	$X_1X_2X_3Y$	Payne, 1912a; Goldsmith, 1916
<i>Rocconata annulicornis</i>	X_1X_2Y	Payne, 1909
<i>Sinea diadema</i>	$X_1X_2X_3Y$	Payne, 1909; Montgomery, 1901, 1906
<i>S. complexa</i>	$X_1X_2X_3Y$	Payne, 1912a
<i>S. confusa</i>	$X_1X_2X_3Y$	" "
<i>S. rileyi</i>	$X_1X_2X_3X_4X_5Y$	" "
<i>S. spinipes</i>	$X_1X_2X_3Y$	" "
Cimicidae		
<i>Cimex lectularius</i>	(4 to 16 X's and a Y; number of X's varies with the individual)	Slack, 1939a, b, c
<i>C. rotundatus</i>	X_1X_2Y	" "
<i>C. städleri</i>	X_1X_2Y	" "
<i>C. columbarius</i>	X_1X_2Y	Darlington, 1939
Homoptera		
Aphididae		
<i>Eucraphis betulae</i>	$X_1X_2X_3X_4$	Shinji, 1931
<i>Stomaphis yanois</i>	X_1X_2	Honda, 1921
<i>Phylloxera caryaecaulis</i>	X_1X_2	Morgan, 1908, 1909a, 1909b, 1912, 1915
<i>Ph. fallax</i>	X_1X_2	Morgan, 1906, 1909b, 1912, 1915
Neuroptera		
<i>Ascalaphus libelluloides</i>	(Number of X's and Y's variable)	Naville & de Beaumont, 1933
Lepidoptera		
<i>Phragmatobia fuliginosa</i> (3 races)	$\begin{cases} XY \\ XY_1Y_2 \\ X_1X_2Y_1Y_2 \end{cases}$	$\left\{ \begin{array}{l} \\ \\ \text{Seiler, 1925} \end{array} \right.$
Diptera		
<i>Drosophila miranda</i>	X_1X_2Y	Dobzhansky, 1935; Dobzhansky & Tan, 1936a, 1936b; McKnight, 1939
<i>D. virilis americana</i>	XY_1Y_2	Hughes, 1939
Coleoptera		
<i>Cicindela</i> spp.	$X_1X_2(?)$	Stevens, 1906, 1909; Goldsmith, 1919
<i>Blaps lusitanica</i>	$X_1X_2X_3X_4Y(?)$	Nonidez, 1914, 1915, 1920 (see also Wilson, 1925)

are as causative agents of evolutionary change, we are not concerned here; they may, however, be very minute sectional rearrangements, differing only quantitatively from the second category. Losses and reduplications of whole chromosomes have probably happened only very rarely in animal evolution, although they have undoubtedly occurred fairly frequently in plants, where polyploidy is much more common, and where aneuploidy is consequently less likely to lead to inviability.

In considering problems of chromosomal evolution in animals we are therefore mainly concerned with the second type of change, namely, sectional rearrangements. These include inversions, translocations, deletions, and duplications. All of them originate as a result of the simultaneous occurrence of two or more chromosomal breaks, the fragments so produced subsequently joining up in a way which is different from the original one. Single breaks do not give rise to sectional rearrangements, since there is only one way in which the two fragments can rejoin, namely, the original one (for apparently freshly broken ends of fragments are incapable of becoming attached to original chromosome ends).

Inversions may be divided into two classes, according to whether the spindle attachment of the chromosome is included in the inverted segment or not; the first kind have been called *pericentric*, the second *paracentric* (Muller, 1940). Translocations are of three types, which may be called *shifts*, *insertional translocations* and *mutual translocations*. In a shift a section of a chromosome is removed to another position in the same chromosome (as a result of three breaks). In an insertional translocation a section of a chromosome is similarly inserted into another non-homologous chromosome (as a result of two breaks in one chromosome and one in another). In a mutual translocation the terminal portions of two chromosomes are interchanged (as a result of two breaks, one in each chromosome). Deletions involve the loss of an interstitial portion of a chromosome, while duplications usually result from crossing-over following shifts and other sectional rearrangements.

Terminal deletions, inversions and translocations, if they occur at all, must be extremely rare. The ends of chromosomes thus possess a number of peculiar properties: (1) they are necessary for the life of the chromosome, since their loss always seems to lead to the disappearance of the chromosome from the set, (2) they are incapable of serving as attachment points for freshly broken ends. These properties have caused Muller to apply the term "telomere" to the chromosome end. He seems to envisage the telomere as a gene or chromomere, but it may equally well be a mere pellicle or limiting membrane which, like the skin of a sausage,

closes the end of the chromosome. Only in the species of *Ascaris* (where the germ-line chromosomes break up into a number of smaller chromosomes in the somatic tissues) does the concept of the "telomere" appear to break down. Here, either the germ-line chromosomes must contain a large number of interstitial telomeres, or potential telomeres; or else the somatic chromosomes are capable of existing without telomeres, or of manufacturing them *de novo*.

Animal chromosomes appear to be of two main types which can be called "V-shaped" and "rod-shaped". A V-shaped chromosome has its spindle attachment somewhere about half-way along its length. In rod-shaped chromosomes the spindle attachment is very near one end (some authorities believe that the spindle attachments of rod-shaped chromosomes are actually terminal, but this seems rather doubtful, although for most purposes they can be considered so). One of the commonest processes in chromosomal evolution is the fusion of two rod-shaped chromosomes to produce a V-shaped one. This almost certainly results from a mutual translocation, but it is not clear whether the V-shaped chromosome so produced has a single spindle attachment or two situated so close together that they function as one, as in the case of *Ascaris megalocephala* (White, 1936).

Most of the changes in chromosome number which have occurred in the course of evolution have almost certainly been the result of sectional rearrangements. If most of a chromosome becomes translocated on to or into another chromosome, the minute region containing the spindle attachment which is left behind will usually be lost from the chromosome set after a few generations if it does not contain any essential genes. This is probably the usual way in which chromosome numbers become diminished.

Evolutionary increases in chromosome number probably come about through duplication of regions containing a spindle attachment. One way in which this can occur is through the formation of small supernumerary chromosomes. Most of the "supernumerary" chromosomes which have been described have probably arisen through the deletion of a large part of a chromosome, the parts containing the spindle attachment and telomere having subsequently joined up to form a chromosome which is a good deal smaller than the original one. Such a "supernumerary" may easily become included in a gamete which also contains the normal unaltered chromosome. In this way the "supernumerary" may be transmitted to subsequent generations and may later become part of the regular chromosome set of the species, having possibly had portions of other chromosomes translocated on to it.

Most of the general laws of chromosomal evolution appear to apply to the sex chromosomes as well as to the autosomes; but the sex chromosomes seem to be to some extent isolated in their evolution from the rest of the chromosome set. This is readily understandable, since any sectional rearrangements involving the sex chromosomes are particularly likely to upset the delicately poised sex-determining mechanism. Thus in many groups of animals where the total number of chromosomes varies very greatly from species to species the sex chromosomes are invariable in size, number and shape.

(3) *Behaviour of sex chromosomes at meiosis*

In order that chromosomes may segregate regularly to opposite poles at meiosis it is usually necessary that they should have undergone pairing at zygotene. Since pairing only takes place between homologous regions it follows that where two different kinds of X 's are present together with a Y (X_1X_2Y mechanism), the Y must contain regions homologous to parts of both the X 's. Conversely, where there is an XY_1Y_2 mechanism the X must contain regions homologous to both the Y 's.

In the vast majority of organisms zygotene pairing is followed by chiasma formation. Thus when the chromosomes segregate at the anaphase of the first meiotic division they are held together, not by the zygotene pairing force, but by the existence of one or more chiasmata between the homologues. There are, however, certain groups of animals which constitute exceptions to this generalization. Thus in male Mantids chiasmata are apparently not formed, but the homologues remain paired until anaphase. In *Drosophila* species although chiasmata are formed between the sex chromosomes none are formed in the autosomes of the male (Darlington, 1934). Conversely, in the Neuroptera and Heteroptera, although chiasmata are formed between the autosomes, none are formed between the X - and Y -chromosomes, which nevertheless regularly segregate to opposite poles (usually at the second division in the Heteroptera instead of at the first, as in other organisms).

Thus in these two orders of insects we have a phenomenon which will be referred to as *determinate disjunction*, that is to say, one chromosome determines the disjunction of another (at either the first or second meiotic division) without there being any direct connexion between them. Any interpretation of the physical basis of *determinate disjunction* must necessarily be speculative, but on Darlington's general theory of mitosis and meiosis it may be imagined to be the result of a rather delicately

poised equilibrium of attractional and repulsional forces inside the developing spindle area at prometaphase.

Whether this be so or not, it is clear that there are several different types of determinate disjunction. Thus in the Heteroptera it is always preceded by a close approximation of the chromosome concerned, while in the Neuroptera the X - and Y -chromosomes which proceed to opposite poles of the spindle never come near to one another at all, attaching themselves near the opposite poles of the spindle in the first place (this type of determinate disjunction has been called "distance conjugation" by Darlington (1937)). Another type of determinate disjunction is met with in those animals which have multiple X 's in the male, but no Y (e.g. *Perla marginata*). This type of determinate disjunction is exactly the reverse of the preceding ones, whereas in the Neuroptera and in many Heteroptera chromosomes which are not physically joined together by chiasmata regularly travel to *opposite* poles, in the X_1X_2 , $X_1X_2X_3$, ... animals several independent chromosomes regularly travel to the *same* pole.

A fourth type of determinate disjunction has been reported by Payne (1912*b*, 1916) in the case of *Gryllotalpa borealis* and appears to be also present in *Drosophila miranda* (see below, § II (10)). Here two chromosomes which are joined together by chiasmata determine the segregation of a third chromosome which is a univalent; the latter always goes to the same pole as one of the first two.

Whether it is really justifiable to apply the same term "determinate disjunction" to all these rather different types of phenomena may be open to doubt. For the present it seems convenient to do so; they all have one main feature in common, the fact that one chromosome determines the segregation of another without there being any direct connexion between them.

In animals in which true pairing between the sex chromosomes has been replaced by a mechanism of determinate disjunction in the heterogametic sex we cannot be sure whether homologous regions exist in the X 's and Y 's. It is to be presumed that chiasma formation takes place between the X -chromosomes in the homogametic sex of such animals, although this point has never been verified by direct observation.

(4) *The methods of investigation*

There are five main methods which are available for the interpretation of problems of chromosomal evolution. In the first place we can simply compare the sizes and shapes of chromosomes as seen at metaphase, and

draw deductions from these. This method is applicable in all cases, but its usefulness is obviously strictly limited—it gives a minimum of information, and any conclusions based solely on this method are rightly open to doubt.

In the second method another criterion besides size and shape is taken into account—heteropycnosis of particular chromosomal regions is studied and used as a basis for drawing conclusions.

In the third method the pairing and chiasma formation of homologous regions at meiosis are studied. In many species all these three methods can profitably be combined; § II (2) of this article will provide an example of the way in which this can be done.

The fourth method consists in the examination of the salivary gland chromosomes—those enormously enlarged chromosomes in which individual regions can be recognized by the banding pattern which is characteristic for them. Not all animals, unfortunately, possess salivary gland chromosomes, in fact they have only been found up till now in the single order Diptera.

The fifth method is the construction of genetical maps of the chromosomes by means of linkage experiments. This method, although it must be regarded as the final “court of appeal” in problems of chromosomal evolution, is necessarily very slow, even in animals like *Drosophila* spp., which are relatively easy to culture and have a short life history. Where all five methods are available we have the ideal conditions for investigating chromosomal evolution; but in many groups of animals in which the fourth and fifth cannot be used it is nevertheless possible to make a number of deductions as to the probable mode of derivation of one chromosome set from another, which, even if not wholly correct in all cases, are nevertheless useful as first approximations to the truth.

II. MULTIPLE SEX CHROMOSOMES IN ORTHOPTERA (SENS. LAT.)

(1) *Evolution of sex chromosomes in the Acrididae*

The Acrididae (locusts and grasshoppers) are a particularly favourable group for studying problems of chromosomal evolution. In the subfamilies Oedipodinae, Truxalinae, and Catantopinae (Cyrtacanthacrinae), the vast majority of the species have a strongly heteropycnotic X-chromosome and eleven pairs of rod-shaped autosomes. This must be regarded as the “standard” chromosome set from which all the aberrant chromosome sets (usually occurring in single species) have been derived by various structural rearrangements.

Whereas the “standard” chromosome set in the Acrididae is an XO

one, a few species have secondarily acquired an XY mechanism through fusion of the X with one member of a pair of autosomes. There can be little doubt that this "fusion" has come about through a reciprocal translocation. The effect of such a translocation will be to make the male of the species XY , the " Y " so created being simply an autosome which is homologous to one limb of a V-shaped X -chromosome, the other limb being the original X which can still be distinguished because it is heteropycnotic at meiosis. A recently arisen $XY:XX$ mechanism of this type will presumably differ from a long-established one in that the " Y " will be more or less completely active, and can undergo crossing-over with the whole of one limb of the X . A recently-arisen Y of this kind may be appropriately referred to as a "neo- Y ."

Clear examples of this kind of transformation were described by McClung (1917) in various species of the genus *Hesperotettix*. Whereas *H. brevipennis* and *H. festivus* have a normal chromosome set (XO in the male), *H. pratense* and *H. speciosus* both have the X fused with one member of a pair of autosomes so that the chromosome number in both sexes is 22 instead of 23 in the male and 24 in the female. There are some indications that the autosome involved in the translocation is not the same in the two species (apparently it is one of the longer ones in *H. speciosus* and one of the medium-sized ones in *H. pratense*). Finally, in *H. viridis* McClung found a large number of different types in the populations he studied, in some of which the X was "free", while in others it was translocated to an autosome. In certain individuals of this species some of the rod-shaped autosomes had fused to form V's, thus increasing the number of chromosomal types found in the species. In one individual a "supernumerary" chromosome was present in addition to the normal chromosomal set.

In the genus *Mermira* some of the species appear to be XO , but *M. bivittata* has become XY through fusion of the X with a rod-shaped autosome as in *Hesperotettix*. An interesting point is that the " Y " in *Mermiria* is not rod-shaped but V-shaped (Fig. 1). Clearly, another structural change in the sex-chromosome mechanism has taken place in addition to the translocation which established an XY condition. The nature of the "extra" region in the " Y " is not clear, but it is probably a duplication of some region present elsewhere in the chromosome set (for this reason it has been labelled *Dup.* in Fig. 1); possibly it arose by fusion of a "supernumerary" with the " Y ". In individuals belonging to what McClung calls the "green variety" of *M. bivittata* the "Dup." region is a good deal shorter than it is in typical individuals.

The structural changes which have taken place in *Hesperotettix* and *Mermiria* have merely converted an XO mechanism into XY ones; they have not led to the establishment of a "multiple" mechanism as defined at the beginning of this article. They have been described here because the principles involved are important for the understanding of the next case that we shall consider.

(2) The case of Paratylotropidia

Paratylotropidia brunneri Scudder is a grasshopper belonging to the group Melanopli of the subfamily Catantopinae (Cyrtacanthacrinae). Its chromosomes have been described by King & Beams (1938). Among the

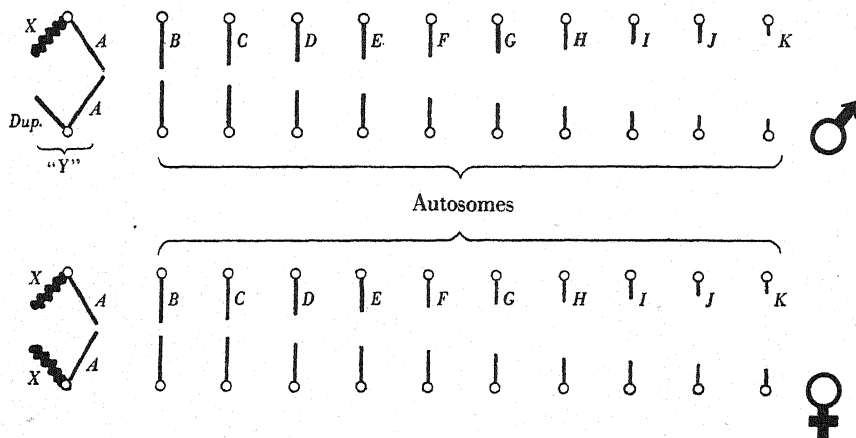


Fig. 1. Diagram of the male and female chromosome sets of *Mermiria bivittata*, as interpreted in the text. Spindle attachments are represented diagrammatically by small circles.

Acrididae it is unique in possessing an X_1X_2Y mechanism. The male diploid set consists of 19 chromosomes, the female one of 20. In both sexes there are four V-shaped chromosomes, two of which represent a pair of autosomes. The other two represent X_1 and the Y in the male, the two X_1 's in the female.

At meiosis in the male a sex-trivalent is formed, in such a manner that X_1 and X_2 pass to the same pole at the first division, the Y to the other (see Fig. 3). X_2 is a rod-shaped chromosome.

The simplest explanation of the facts would appear to be the following: the original X has fused at its proximal end with a rod-shaped autosome (as in *Hesperotettix* and *Mermiria*) and the other member of this autosomal pair has fused at its proximal end with another autosome. On this view the "Y" has two arms, one of which is homologous with X_2 , the

other with one arm of X_1 . This is the interpretation adopted by King & Beams. A careful examination of the authors' figures suggests, however, that it is probably incorrect and that a slightly more complicated series of structural changes has taken place. Figs. 7-9 of King & Beams' paper which show the sex-trivalent at diakinesis are particularly interesting because they show the degree of heteropycnosis in the different regions very clearly. Since the details are essentially the same in all three figures it seems clear that one can use the degree of heteropycnosis as an indication of homology or non-homology.

Starting at one end of the sex-trivalent we find that the original X -chromosome (forming the "left" arm of X_1) is densely heteropycnotic. The "right" arm of X_1 (called " a " by King & Beams) is much less heteropycnotic, except for its tip, which is joined to the tip of the "left" arm of the Y by a terminal chiasma. Both limbs of the Y are densely heteropycnotic, although possibly a little less so than the left arm of X_1 . X_2 (called " b " by King & Beams) is a non-heteropycnotic chromosome which is joined to the tip of the "right" arm of the Y by a terminal chiasma.

We can now see why King & Beams's interpretation must be doubted. The two limbs of the Y cannot be homologous to X_2 and the right arm of X_1 respectively, because they are heteropycnotic, whereas the arms called " a " and " b " by King & Beams are not. It is, however, clear that the tip of the right arm of X_1 (labelled B in Fig. 2) is homologous to the tip of the left arm of the Y , since a chiasma must be formed in this region; in agreement with this we find that the B region is heteropycnotic. Similarly the tip of X_2 (labelled A in Fig. 2) must be homologous to the tip of the right arm of the Y , since here also a chiasma is formed.

These facts lead to the following interpretation of the origin of this strange sex-trivalent (Fig. 2). The right limb of X_1 and X_2 are probably in the main homologous to one another, but the former has received the region B as a result of a mutual translocation. The two limbs of the Y are in the main homologous to one another (as is suggested by their heteropycnosis); thus on this interpretation the " Y " of *Paratylotropidia* is analogous to the "attached- X " in *Drosophila melanogaster* (Morgan, 1938); whether it has arisen in a similar manner cannot be decided at present. The right limb of the Y does, however, differ from the left in that its tip has been replaced by the region A (which must be very small, since it is difficult to make out in King & Beams's figures, except possibly in their Fig. 9).

Such a sex-trivalent as that postulated above could have arisen from

the usual grasshopper chromosome set as a result of three successive translocations: (1) a translocation whereby the X is joined at its proximal end to an autosome A , (2) a mutual translocation whereby the tip of this autosome is interchanged with the tip of another autosome B , (3) a translocation whereby the two B -chromosomes become fused at their proximal ends. This appears to be the simplest interpretation, but other more complex ones are not impossible—inversions may also have taken place in any of the chromosomes concerned. If the "triple translocation hypothesis" is substantially correct, it is still not possible to decide

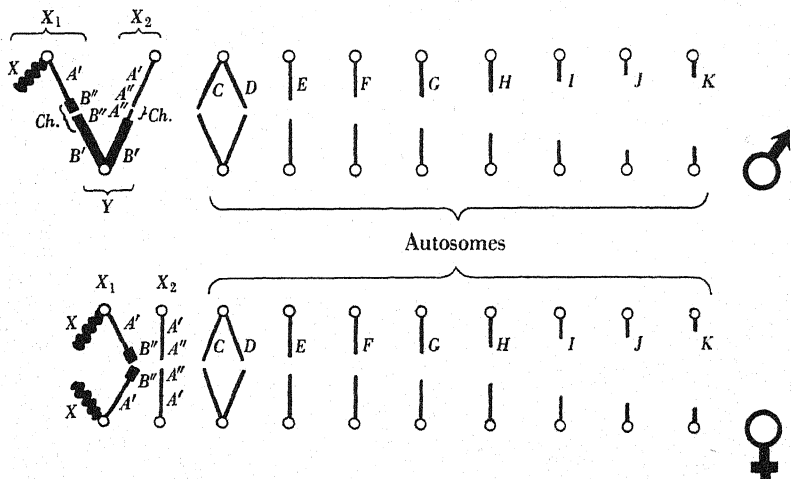


Fig. 2. Diagram of the male and female chromosome sets of *Paratytopotridia brunneri*, as interpreted in the text. The heteropycnotic parts of X_1 and Y are represented as thicker than the non-heteropycnotic parts and the original X -chromosome is distinguished by a wavy outline. The regions of the sex-trivalent where chiasmata are formed are labelled *Ch.*

whether all three events took place simultaneously (i.e. in the same individual grasshopper) or successively, and, if the latter, in what order.

Such a mechanism as the sex trivalent of *Paratytopotridia* could only function if chiasma formation were restricted to the distal ends of the chromosomes. Otherwise the right limb of X_1 and X_2 (i.e. the two A' regions) would sometimes pair and undergo chiasma formation—which would almost certainly lead to non-disjunction. It is therefore interesting to note that in some of the chromosomes of *Paratytopotridia* chiasma formation does appear to be restricted to the distal ends (as is shown by the high frequency of "rod-bivalents").

King & Beams did not study meiosis in the female *Paratytopotridia*, but it seems probable that the two X_1 's form a bivalent and that the two

X_2 's do likewise. If the present author's interpretation is correct, however, then the female animal must have the A' region present four times (twice in X_1 and twice in X_2) and it must lack the B' region altogether (see Fig. 2).

Now according to the hypothesis the B' and B'' regions were originally parts of an autosome which was presumably present in both sexes. It is consequently rather a drastic assumption to suggest that the B' region has been entirely lost from the chromosome set of the female. But since the regions in question are strongly heteropycnotic there is every probability that they are essentially inert. It is clear that losses of chromosomal material must have taken place in the course of evolution; probably in most cases the loss has been gradual, very minute regions being lost by successive deletions; in this case the loss appears to have been sudden.

The X_1X_2Y mechanism in *Paratylotropidia* appears superficially similar to that found in some of the Mantidae (see next section). But if the above interpretation is correct in principle it has arisen in a totally different way. Both clearly arose from XO mechanism but whereas the Mantid sex-trivalent probably owes its origin to a single translocation in a species which already possessed V-shaped chromosomes (see Fig. 4) the *Paratylotropidia* one probably arose by several successive translocations in a species which must have originally had rod-shaped chromosomes. In the X_1X_2Y Mantids both X_1 and X_2 contain parts of the original X , whereas in *Paratylotropidia* only the X_1 appears to contain the original X ,

(3) Multiple sex chromosomes in Mantids

The majority of the Mantoidea are probably $XO:XX$, the X -chromosome being V-shaped. This is the case in at least the following genera: *Callimantis*, *Iris*, *Ameles*, *Acontista*, *Empusa*, *Gongylus*, *Miomantis* (White, 1938, and later unpublished work). On the other hand, a group of at least six genera (*Mantis*, *Tenodera*, *Paratenodera*, *Hierodula*, *Sphodromantis* and *Stagmomantis*) have an X_1X_2Y mechanism (Oguma, 1921; King, 1931; Asana, 1934). These genera are taxonomically close to one another, so that it is probable that their sex-chromosome mechanism had a common origin, probably in tertiary times.

The probable origin of the X_1X_2Y mechanism is shown in Fig. 3. All three chromosomes of the sex-trivalent are V-shaped. The "left" arm of X_1 and the "right" arm of X_2 show negative heteropycnosis in the spermatogonial divisions; it is therefore almost certain that they represent the two limbs of an originally single and V-shaped X such as we still find in other Mantids. A single reciprocal translocation between a V-shaped

X and a V-shaped autosome (both breakage points being very near the spindle attachments) could have given rise to the X_1X_2Y mechanism. In this way an "AB" autosome would be converted into a "Y". By pairing with X_1 and X_2 the latter ensures that they shall both pass to the same pole at the first meiotic division in the male.

Although this was probably the origin of the X_1X_2Y mechanism, the "Y" of the modern species has almost certainly undergone a great deal

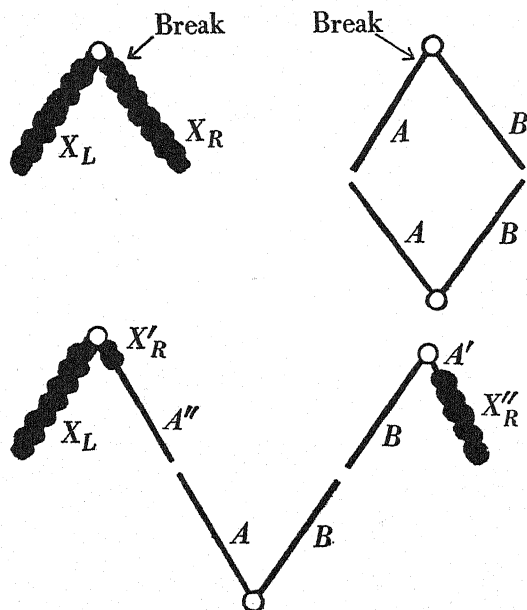


Fig. 3. Diagrams illustrating the origin of the X_1X_2Y mechanism in Mantids. The upper figure illustrates the condition before the translocation, the lower one after it. The original X and its parts after translocation are represented with wavy outlines. X_R and X_L are the "right" and "left" arms of the original X , X'_R and X''_R the two portions of X_R after breakage. Similarly A' and A'' are the two portions of the A limb and of the AB autosome.

of evolutionary change since the time when it was merely an "AB" autosome. In *Mantis religiosa* it is a large V-shaped chromosome whose two arms are about the same length as the "right" arm of X_1 and the "left" arm of X_2 . This may represent the original condition. In *Paratenodera sinensis* the Y is still V-shaped, but the two arms are unequal in length (this is not clear in King's figure, but is very obvious in the present author's preparations). In *Hierodula* and *Stagmomantis* the Y is very small.

If the X_1X_2Y mechanism really arose in a single species, then the different forms of Y which are found in the six genera must be the result

of evolutionary divergence. Alternatively, the X_1X_2Y mechanism may have arisen several times in a number of taxonomically related genera; but this seems unlikely.

It is worth noting that the X_1X_2Y mechanism appears to be a mechanically unsatisfactory one, since non-disjunction of the sex chromosomes seems to be not infrequent. The sex trivalent is very large and its orientation on the spindle is apparently effected with difficulty. Any reduction in size of the Y-chromosome (such as seems to have occurred in *Hierodula* and *Stagmomantis*) would probably render the sex trivalent less "clumsy" in its meiotic movements.

Whatever changes have taken place in the Y, it is clear that the ends have remained homologous to the tip of the "right" arm of X_1 and of the "left" arm of X_2 , so that pairing can still take place in these regions.

(4) *Multiple sex chromosomes in earwigs*

Most of the Dermaptera are probably $XY:XX$, but in two genera (*Anisolabis* and *Forficula*), belonging to different families, an X_1X_2Y mechanism appears to have been evolved. *Anisolabis maritima* and *A. annulipes* both show this type of "multiple" mechanism (Morgan, 1928), but there does not appear to be any possibility of deciding how it arose. A sex trivalent is formed at meiosis, but since the three chromosomes of which it is composed are of approximately the same size they cannot be distinguished with certainty. From the author's figures it is not possible to make out any special heteropycnotic regions as in the Mantids previously discussed; neither is it possible to tell whether one is dealing with rod-shaped or V-shaped chromosomes.

In *Forficula auricularia* there appear to be two kinds of males in the population (Morgan, 1928), one kind being XY (with 24 chromosomes in the diploid set), the other X_1X_2Y (with 25 chromosomes). Again, no conclusion is possible as to the origin of the "multiple" mechanism, although it is clear that both kinds of individuals have 22 autosomes.

III. MULTIPLE SEX CHROMOSOMES IN THE *RHYNCHOTA*

(1) *Sex chromosomes in Reduviids and in some other Heteroptera*

In the Heteroptera compound sex chromosomes have been described in the families Galgulidae (Gelastocoridae), Nepidae, Coreidae, Pentatomidae, Cimicidae, and Reduviidae. In the Belostomatidae, Capsidae, Lygaeidae, Nabidae, Notonectidae, and Pyrrhocoridae no multiple sex chromosomes have been reported up to now.

It is unfortunate that the processes of chromosomal evolution in the

Heteroptera are not nearly so easy to analyse as those of the Orthoptera. Not only are the chromosomes on the whole very much smaller, but in addition they are highly condensed at mitosis and meiosis, so that it is impossible to determine the positions of the spindle attachments. We are thus unable to say whether any particular chromosome is of the rod-shaped or the V-shaped type. In fact the very existence of spindle attachments in the Heteroptera is a matter of inference, since they have not been directly observed in this group. For this and other technical reasons it is not possible to interpret the sex-chromosome mechanisms of the Heteroptera in the same detailed way that has been possible in the Orthoptera.

Another peculiarity of the chromosomes in this group is that, whereas in most organisms all the spindle attachments in the chromosome set appear to behave in the same way at mitosis and meiosis, so that one could consider them as "homologous", in many Heteroptera the spindle attachments in the sex chromosomes seem to behave differently from those in the autosomes. Thus those in the sex chromosomes divide at the first meiotic division in the male, so leading to an "equational" separation of the X and Y, while those in the autosomes do not divide until the second division. This may mean that the spindle attachments in the sex chromosomes are really different from those in the autosomes, or it may be that the time of division of the spindle attachment is influenced by the rest of the chromosome, or at any rate that part of it lying near the spindle attachment. In a few of the Heteroptera (e.g. *Archimerus*) the spindle attachments in the sex chromosomes behave normally, i.e. they divide at the same time as those in the autosomes. It will be seen from Table I that a rather large number of Reduviids have multiple X-chromosomes. Of the sixteen species investigated by Payne (1909, 1910, 1912) and Montgomery (1901, 1906) three are XY in the male, three X_1X_2Y , six $X_1X_2X_3Y$, while two are $X_1X_2X_3X_4X_5Y$. Most, but not all, of the species with multiple X's belong to the subfamily Zelinae.

In all these species meiosis in the male appears to take place in the usual Heteropteran manner; that is to say the sex chromosomes divide equationally in the first division and come together in the middle of the spindle at the second division, the Y then going to one pole, the X to the other.

One interesting feature which may be noted, although its significance is not clear, is that the Y-chromosome in some of the species (e.g. *Prioidus cristatus*) is a good deal larger than the individual X-chromosomes, or even larger than all of them put together (in *Acholla multispinosa*).

It would appear from Payne's accounts that the sex chromosomes in any particular species of Reduviid are constant in number, instead of varying from individual as they do in *Cimex lectularius* and *Ascalaphus libelluloides* (see below). But it is not clear that he used a sufficiently large number of individuals in his work to be sure that this is always so.

Three lines of evidence suggest that the multiple X 's of Reduviids represent reduplicated portions of an originally single X , rather than autosomes or portions of autosomes which have become involved in the sex-determining mechanism. In the first place they are apparently all heteropycnotic during the prophase of the first meiotic division, the autosomes being non-heteropycnotic. Secondly their spindle attachments behave like those of Heteropteran sex chromosomes, dividing at the first meiotic division, instead of at the second as those of the autosomes do. Lastly it is worth noting that all the five species of *Sinea* investigated by Payne have the same number of autosomes (twelve pairs) while the sex-chromosomes may be $X_1X_2X_3Y$ or $X_1X_2X_3X_4X_5Y$. If the extra X 's were of autosomal origin we should rather expect to find that the number of autosomes would be correspondingly reduced in the $X_1X_2X_3X_4X_5Y$ species.

It is thus fairly clear that the "multiple" mechanisms in the Reduviids are fundamentally different in nature from those met with in the Orthoptera, which are the result of mutual translocations between the X and an autosome. The Reduviid type of "multiple" mechanism (which depends on a special kind of determinate disjunction) could probably not exist in a species where the sex chromosomes form chiasmata in the heterogametic sex. If it did $X_1X_2X_3, \dots$, being all in part at least homologous, would sometimes pair with one another and form chiasmata in such a way that they would go to opposite poles at the first meiotic division (which would upset the sex-determining mechanism completely).

Apart from the Reduviid cases, various other "multiple" mechanisms have been described in the Heteroptera, but it is not always clear how they have arisen. In *Galgulus (Gelastocoris) oculatus* there is an $X_1X_2X_3X_4Y$ mechanism which appears to behave at meiosis like those of the Reduviids, and is therefore presumably of the same type (Payne, 1909, 1912a). A similar type of mechanism has been reported by Steopoe (1925) for *Nepa cinerea*. In the Pentatomid bug, *Thyanta calceata*, there is an X_1X_2Y mechanism, while the very closely related species *Th. custator* has an XY pair (Wilson, 1909b, 1911). That extensive chromosomal rearrangements have taken place in the recent ancestry of these

two species is suggested by the fact that the former has a diploid number of 28 (in the female) while the latter has only 16 chromosomes.

In *Syromastes marginatus* (Coreidae) there is a "multiple" mechanism which is so far unparalleled in the Heteroptera, the male being X_1X_2 , the female $X_1X_1X_2X_2$. A Y is thus entirely lacking. In the male X_1 and X_2 remain very close together throughout meiosis, although there is no evidence that any chiasmata are ever formed between them. They divide equationally in the first meiotic division, but go undivided to the same pole at the second one (Wilson, 1909a, b).

(2) Sex chromosomes in Cimex

Slack (1939 a, b, c) has described the sex chromosomes in three species of bed bugs, *Cimex rotundatus*, *C. städleri* and *C. lectularius*. The first two possess 14 pairs of autosomes and an X_1X_2Y mechanism. Meiosis in the male is of the usual Heteropteran type, that is to say, the sex chromosomes do not form any chiasmata; they divide "equationally" in the first division and come together momentarily in the centre of the metaphase plate at the second division, then segregating in such a manner that X_1 and X_2 go to one pole, the Y to the other.

The conditions in *C. lectularius* are a good deal more complicated. There are only 13 pairs of autosomes (i.e. one pair less than in *C. städleri* and *C. rotundatus*) but a variable number of supernumerary chromosomes is associated with the sex-chromosome mechanism. The nature of these supernumerary elements is not clear; but it seems likely that two of the largest of them correspond to the X_1 and X_2 of *rotundatus* and are to be regarded as true sex chromosomes. The total number of non-autosomal chromosomes apart from the Y varies from 4 to 16 in different individuals. These chromosomes and the Y all divide "equationally" at the first division in the male, but they often lag on the spindle at anaphase, so that unequal numbers of them are transmitted to the two poles. At the second division all the non-autosomal chromosomes take up a central position, in the middle of the circle of autosomes. Usually the Y goes to one pole and all the rest to the other pole; but there seems to be a good deal of irregularity, so that frequently some of them accompany the Y.

Although it is clear that the sex determination in *Cimex* spp. depends on determinate disjunction of the same type as that found in Reduviids, it is not entirely clear how *C. lectularius* has acquired the large number of supernumerary chromosomes that most individuals seem to carry. Most probably they are essentially inert fragments of X_1 and X_2 which

contain a spindle attachment and one or two telomeres, but lack the active regions of X_1 and X_2 ; not all the supernumeraries are homologous, since they are of several different sizes.

(3) Multiple sex chromosomes in Aphids

Most species of Aphids are XO in the male sex, but Morgan (1906, 1909a, b, 1912, 1915) has shown that two species of *Phylloxera* have an X_1X_2 : $X_1X_1X_2X_2$ sex-chromosome mechanism, one of the X's being very much smaller than the other. Honda (1921) has reported that *Stomaphis yanois* is also X_1X_2 in the male. According to Shinji (1931) the Aphid *Euceraaphis betulae* is $X_1X_2X_3X_4$ in the male. Since there is no Y in any of these cases the mechanisms are similar in principle to that found in *Syromastes marginatus*. It does not seem possible to arrive at any definite conclusion as to the origin of the multiple X's in Aphids, but they have most probably arisen in the same way as those of the Reduviids discussed above.

IV. THE CASE OF *ASCALAPHUS LIBELLULOIDES*

Naville & de Beaumont (1933) have described an interesting case of multiple sex chromosomes in the Neuropteran *Ascalaphus libelluloides* Schaeff. In general the facts seem to be similar to those in *Cimex lectularius*. There are 20 autosomes which form 10 bivalents at meiosis in the male. The number of sex chromosomes appears to be highly variable; thus three female animals had diploid numbers of 22, 23 and 24 respectively. It seems likely that both the X and the Y may be represented by several chromosomes. It is unfortunately not possible to distinguish X elements from Y elements at the first meiotic division, but from the authors' figures it seems improbable that chiasmata are formed between the sex chromosomes in the male. Thus at the first anaphase very variable numbers of sex chromosomes may go to the two poles. If we represent the numbers going to the "North" and "South" poles as the numerator and denominator of a fraction, the following types of anaphases were seen: $\frac{1}{1}, \frac{1}{2}, \frac{2}{2}, \frac{2}{3}, \frac{2}{4}, \frac{2}{6}, \frac{3}{5}, \frac{4}{5}, \frac{5}{6}$. In some individuals the chromosome set appeared to be the same in all the cells of the testis, while in others it appeared to vary (thus anaphases of the following types: $\frac{2}{4}, \frac{2}{6}, \frac{3}{5}, \frac{5}{4}, \frac{5}{6}$ were all found in the same individual).

While the details of this case are by no means clear, it appears likely that the multiple sex chromosomes result from a duplication of parts of the originally single X and Y chromosomes. Other species of the genus

Ascalaphus appear to have a simple $XY:XX$ mechanism (Naville & de Beaumont, 1936).

V. THE CASE OF *PERLA MARGINATA*

Whereas *Perla immarginata* Say has an $XY:XX$ mechanism (Nakahara, 1919), the species *P. marginata* Panzer appears to have an $X_1X_2:X_1X_1X_2X_2$ mechanism. That is to say, there is a multiple sex-chromosome system, but there is no Y . Junker (1923), who studied this case, reports that at the first meiotic division in the male X_1 and X_2 remain very closely associated to one another, and pass undivided to the same pole at the first meiotic division. It appears likely that both sex chromosomes have a homologous region which causes them to pair, but that they are prevented from forming a chiasma by their heteropycnosis, as happens in tetraploid spermatocytes in some cases (White, 1933). In any case it is clear that there is here a mechanism of determinate disjunction which is very different from that which exists in animals like the Reduviids, which have a Y -chromosome. It would be interesting to know whether the four sex chromosomes of the female form two bivalents at meiosis, or whether they sometimes form a quadrivalent. Unfortunately Junker did not work on the meiosis of the female.

VI. THE CASE OF *PHRAGMATOBIA FULIGINOSA*

In the Lepidoptera it is, of course, the female sex which is heterogametic, the males being XX , the females XY . Seiler (1925) has reported that in the species *Phragmatobia fuliginosa* there are three "races". One of these is simply $XX:XY$; the second is $XX:XY_1Y_2$; the third can be designated by the formula $X_1X_1X_2X_2:X_1X_2Y_1Y_2$. Probably X_2 and Y_2 are largely or entirely homologous. This case has been discussed by Darlington (1937); but the exact relationship of the three races to one another is not entirely clear. Darlington believes that the $XX:XY$ race is the original one, and that in the other two "fragmentation" of the X - and Y -chromosomes has taken place. But it would also be possible to regard the third race as the original one, and the others as races in which one or both members of a pair of autosomes had become translocated to the X and Y . In this case the third race could be designated $XXAA:XYAA$, the second $\widehat{XA} \widehat{XA}:\widehat{XA} YA$ and the first $\widehat{XA} \widehat{XA}:\widehat{XA} \widehat{YA}$ (the yoke sign above the letters indicating that fusion or translocation had taken place).

VII. MULTIPLE SEX CHROMOSOMES IN THE COLEOPTERA

The majority of the Coleoptera are XY or XO in the male sex. In *Blaps lusitanica* (Tenebrionidae), however, there appears to be a complex sex-chromosome mechanism. The facts have been described by Nonidez (1914, 1915, 1920). In the male diploid set there are apparently 35 chromosomes. Two kinds of sperms are formed, with 16 and 19 chromosomes respectively. It appears probable that there is an $X_1X_2X_3X_4Y$ or $XY_1Y_2Y_3Y_4$ mechanism, although Nonidez interpreted the matter differently. From his figures it appears possible that the five sex chromosomes consist of two pairs of homologues and an odd one; in that case the mechanism could, perhaps, best be represented by the formulae: $X_1X_1X_2X_2Y$ (δ) and $X_1X_1X_1X_1X_2X_2X_2X_2$ (φ), or alternatively by the following: $XY_1Y_1Y_2Y_2$ (δ) and XX (φ). Since Nonidez did not work on the chromosomes of the female it is difficult to choose between the various possible interpretations. Apparently segregation of the five sex chromosomes into a group of four and an odd one takes place at the first meiotic division, but a re-investigation of the chromosome cycle in this species would be necessary in order to re-interpret its meiotic mechanism along modern lines. In various species of *Cicindela* Goldsmith (1919) has reported a complex sex-chromosome mechanism (probably of the X_1X_2 type); but the details are not clear.

VIII. SEX CHROMOSOMES IN THE GENUS *DROSOPHILA*(1) *The case of Drosophila miranda*

Drosophila miranda Dobzh. is the only species of the genus which is known to possess a multiple sex-chromosome mechanism. It was originally discovered by Dobzhansky (1935), and has been subsequently studied by Dobzhansky and Tan (1936 *a, b*) and by McKnight (1939). Since in this case the salivary gland method has been available for the analysis the nature of the sex-chromosome mechanism has been worked out much more completely than in those animals (e.g. *Paratylotropidia*) where, in the absence of "salivary chromosomes" less reliable methods of analysis have had to be employed. The facts, as elucidated by Dobzhansky, Tan and McKnight are, briefly, as follows:

Drosophila miranda belongs to the same section of the genus as *D. pseudoobscura* and can be crossed with it. The latter species is $XY:XX$; its X and Y are both V-shaped chromosomes, although the Y varies in shape in the different geographical races into which the species can be split up. Besides the sex chromosomes *D. pseudoobscura*

possesses three pairs of rod-shaped autosomes (II, III and IV) and a pair of minute dot-like chromosomes (V) similar to the IVth chromosome of *D. melanogaster* (see Fig. 4).

The chromosome set in the female *miranda* is very similar to that of the female *pseudoobscura*; in the male *miranda* there are X- and Y-chromosomes which resemble those of *pseudoobscura* (except that the Y of *miranda* always has two arms of about the same length, instead of varying in shape as it does in *pseudoobscura*). In the male *miranda*, however, the IIIrd chromosome is unpaired, so that the diploid set consists of 9 chromosomes instead of 10 as in the female (Fig. 6). Genetic as well as cytological evidence has shown that this unpaired IIIrd chromosome always goes to the same pole as the X at the first meiotic division in the male.¹ For this reason it must be regarded as an autosome which has become part of the sex-chromosome mechanism and may be designated X_2 (the "true" X, i.e. that corresponding to the X of *pseudoobscura*, being referred to as X_1).

The origin of the *miranda* mechanism can be inferred with a high degree of probability. It was at first thought that one of the IIIrd chromosomes had simply been lost from the chromosome set of the male, that is to say that the male *miranda* had suddenly become haploid for this autosome at some stage in its evolution. McKnight has shown, however, that the Y of *miranda* contains a number of euchromatic (i.e. genetically active) regions which are homologous to parts of the X_2 chromosome. Mutations situated in these euchromatic parts of the Y are, however, inherited solely in the male line, since there is never any crossing-over between the Y and X_2 .

It thus appears that the male *miranda* is not, after all, haploid in respect of all the genes in the IIIrd chromosome, since many or most of them are also carried in the Y. McKnight has made the very plausible suggestion that the first step in the origin of the *miranda* mechanism was a translocation whereby most of one of the IIIrd chromosomes was transferred to the Y. He thinks that this part of the IIIrd chromosome, having been attached to or inserted into the Y, was then broken up into a number of short sections and distributed at intervals along the length of the Y in such a way that there was an alternation of euchromatic and heterochromatic regions (a series of inversions would lead to this result).

The meiotic behaviour of the sex chromosomes in *pseudoobscura* has been described by Darlington (1934) and is summarized diagrammatically

¹ Occasionally primary non-disjunction leads to the X and Y going to the same pole, the IIIrd chromosome going to the other pole.

in Fig. 4. It would appear that in the male a pair of "reciprocal" chiasmata is regularly formed between the X and Y , on the same side of the spindle attachment; these chiasmata are the only ones formed in the male, there being no crossing-over in the autosomes. This meiotic mechanism is a very anomalous one, but is apparently found in most of the "higher" Diptera. The existence of chiasma-formation between the

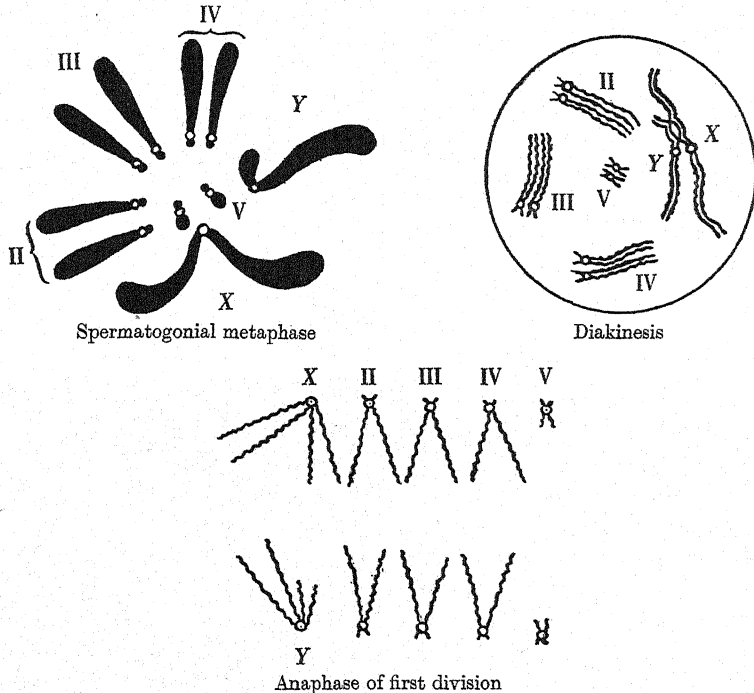


Fig. 4. Diagrams illustrating the main phases of meiosis in the male *Drosophila pseudoobscura* as interpreted by Darlington (1934).

X and Y indicates that they have a short homologous region around the spindle attachments.

In *miranda* the X_1 and Y appear to behave in the same manner as in *pseudoobscura*; X_2 neither pairs nor forms chiasmata with any part of the X_1Y bivalent; that is to say it behaves as a univalent throughout meiosis. Nevertheless it always segregates to the same pole as the X_1 chromosome at the first division (Fig. 6). We thus have in *D. miranda* a mechanism of *determinate disjunction*, similar in principle to that reported by Payne (1916) for *Gryllotalpa borealis*. It is somewhat difficult to imagine what physical mechanism is responsible for this determinate dis-

junction; but, whatever the cause, determinate disjunction certainly exists in a few organisms.

The sex-chromosome mechanism of *Drosophila miranda* is thus in many respects unique. It is an X_1X_2Y mechanism, but it bears no real similarity to the X_1X_2Y mechanisms in *Paratylotropidia* and Mantids. The latter have originated from $XO : XX$ organisms, while *D. miranda*

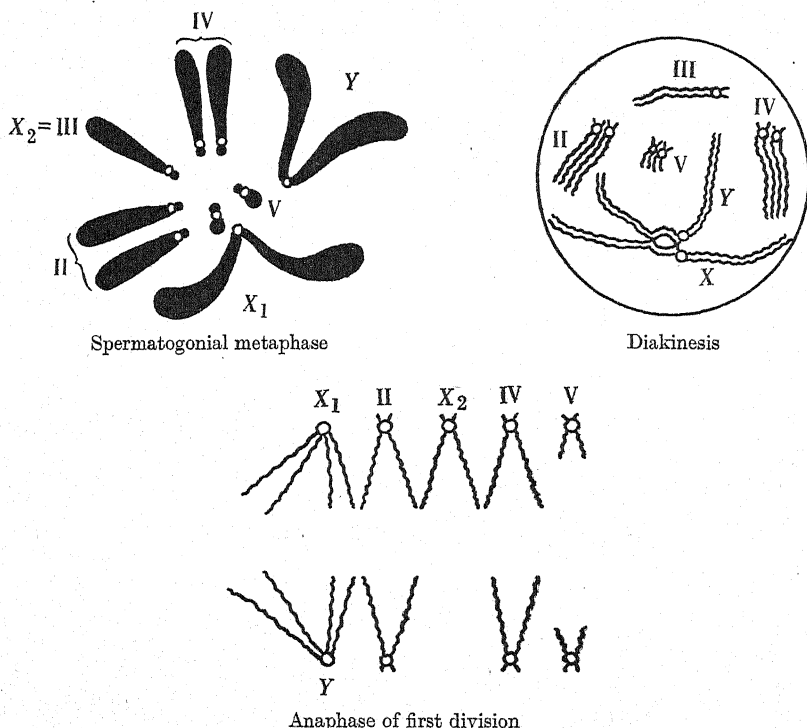


Fig. 5. Diagrams illustrating the main phases of meiosis in the male *Drosophila miranda* as interpreted by Dobzhansky (1935).

has arisen from an $XY : XX$ species. Moreover, the efficiency of the orthopteran mechanisms depends on chiasma-formation at meiosis, while that of the *miranda* mechanism depends partly on chiasma formation (between X_1 and Y), partly on determinate disjunction (between X_2 and the X_1X_2Y bivalent). In its meiotic mechanism *D. miranda* thus lies midway between those organisms whose sex-determining mechanism depends exclusively on chiasma formation (e.g. Orthoptera) and those where it depends on determinate disjunction only (e.g. Reduviids, *Perla*).

(2) *Rod-shaped and V-shaped X-chromosomes*

In some species of *Drosophila* the *X* is a rod-shaped chromosome (as it is in *D. melanogaster* and *D. virilis*); in other species (such as *D. pseudoobscura*) it is a *V*. Genetical analysis suggests that one limb of the *X* in *pseudoobscura* is approximately homologous to the "left" arm of the IIIrd chromosome of *melanogaster* (the arm which is referred to as "*D*" in Muller's (1940) terminology). Actually the sequence of the genes in the *XR* arm of *pseudoobscura* is in many cases different from their sequence in the "*D*" arm of *melanogaster*; but the genes themselves are sometimes clearly homologous—thus proving that the two chromosome arms have a common origin, although repeated paracentric inversions and other structural changes have altered the gene sequence considerably.

If it be true that one arm of the *X* in *pseudoobscura* is homologous to an autosomal arm in *melanogaster*, then, as McKnight (1939) points out, *pseudoobscura* must have become haploid in the male for a chromosome arm which is present in the diploid state in both sexes of *melanogaster*. We have at present no means of ascertaining with certainty how the *pseudoobscura* condition arose. McKnight has, however, made the suggestion that haploidy of the "*D*" arm did not arise suddenly; he thinks that in the ancestor of *pseudoobscura* one of the two "*D*" arms became translocated to the *X*, the other to the *Y*. The arm thus transferred to the *Y* he thinks became gradually inert. This suggestion is plausible in view of McKnight's own work on *D. miranda*. It appears probable to the present author that the *pseudoobscura* condition is derived from a chromosome set similar to that found in *D. virilis*, where all the chromosomes (including the *X* and *Y*) are rod-shaped (though it is not, of course, suggested that the actual ancestor was *D. virilis*, or even a species closely related to it). In such a species "*D*" would be an independent rod-shaped autosome, not one arm of a *V*-shaped chromosome. Two translocations of the type postulated by McKnight (involving the transference of the whole or almost the whole of "*D*") would then give rise to the *pseudoobscura* condition.

These two translocations may have occurred simultaneously or successively. In the latter case (which is more probable on *a priori* grounds) a complex sex-chromosome mechanism (of the X_1X_2Y or XY_1Y_2 type) must have existed in the interval between the two translocations (see Fig. 6).¹ That such a condition does in fact exist in *D. miranda* proves that

¹ The second "translocation" may quite possibly have been the result of crossing-over between *X* and *Y* (or between *X* and Y_1) in the neighbourhood of the spindle attachment.

this kind of thing is not a mechanical or physiological impossibility in the genus *Drosophila*. *D. miranda* cannot, of course, be the "missing link"

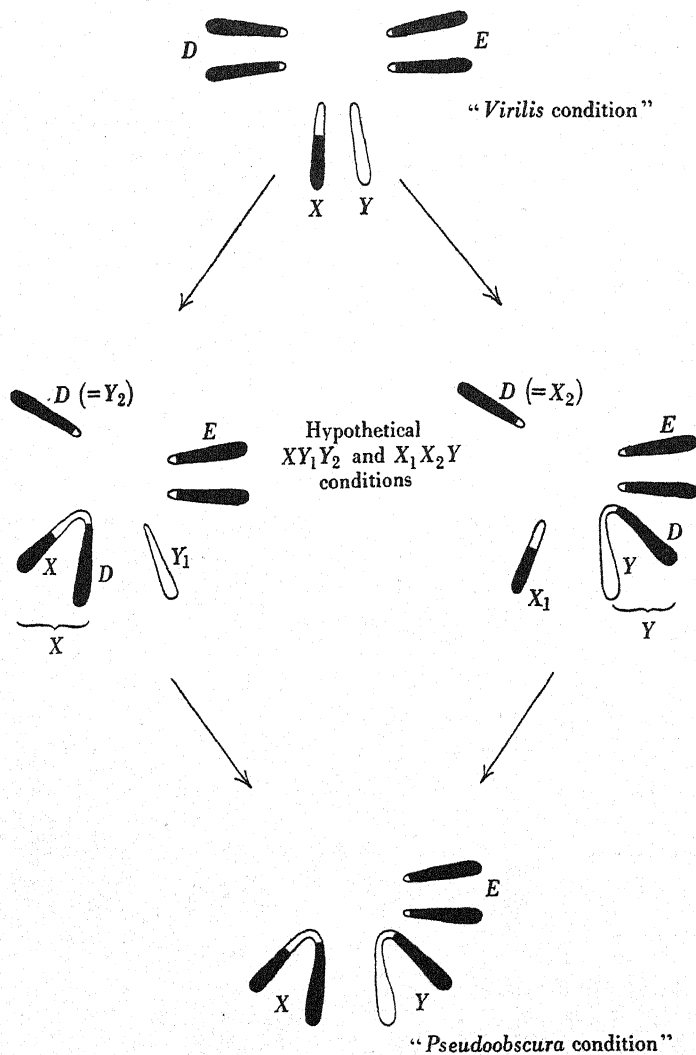


Fig. 6. Diagrams illustrating a possible mode of origin for the sex chromosomes of *Drosophila pseudoobscura*. Only two pairs of autosomes ("D" and "E") are shown, for the sake of simplicity. The inert regions are white, active regions black. Further explanation in the text.

between the "virilis condition" and the "pseudoobscura condition", since it is clearly a later derivative of the latter. In any case the fact that in

miranda both X_1 and Y are V-shaped precludes it from being the "missing link". Perhaps, however, it is not too speculative to suggest that *miranda* is at the present time in a condition analogous to that of the "missing link" which we have postulated; if the X_2 of *miranda* were to be translocated to X_1 the species would revert to an XY condition.

Until recently the present author was inclined to regard complex sex-chromosome mechanisms as being, for the most part, evolutionary cul-de-sacs; but if the above argument is substantially correct they may in some cases have been important intermediate steps between one kind of XY mechanism and another.

In some species of *Drosophila* (*D. hydei* and *D. repleta*) there exist V-shaped X -chromosomes one arm of which is heterochromatic (and consequently "inert"). What relationship this type of X bears to the *melanogaster* and *pseudoobscura* X 's cannot be decided at present; but since in these two species the Y is rod-shaped it is possible that the inert arm of the X has been received as a translocation from a V-shaped Y .

IX. MULTIPLE SEX CHROMOSOMES IN THE ARACHNIDA

Painter (1914) described an X_1X_2 condition in the males of a number of species of spiders. His account has recently been confirmed by Hard (1939) for one species. Both X -chromosomes go to the same pole at the first meiotic division; there do not appear to be any chiasmata between them, but they lie closely parallel to one another. Thus a mechanism of determinate disjunction is present which seems to be of the same type as that met with in *Syromastes* and in *Perla marginata*.

There seem to be a number of points worth noting. In the first place the species studied by Painter belong to eleven different families, and in all of them the sex chromosome mechanism was of the same type (although the number of autosomes varied from 9 to 21 pairs). This is a very different state of affairs from that found, for example, in the Reduviids. The spiders seem, in fact, to be the only large group all the members of which possess the same "multiple" mechanism. This suggests that the X_1X_2 system in the order is of great antiquity and has not undergone any essential changes in the course of its evolution (the spiders as a group go back in geological history as far as the Carboniferous).

Another fact which may be significant is that X_1 and X_2 are always of exactly the same length and are both heteropycnotic. It almost looks as if we were dealing, not with an $X_1X_2 : X_1X_1X_2X_2$ mechanism, but with an $XX : XXXX$ one; or, in other words, that " X_1 " and " X_2 " are homologous throughout their whole length but are prevented from forming

chiasmata in the male by their heteropycnosis. If this is so, then the method of sex determination in spiders is unique and unlike any of the "multiple" mechanisms we have hitherto considered.

X. MULTIPLE SEX CHROMOSOMES IN THE NEMATODA

Among the Nematodes a number of species which show multiple sex chromosomes have been studied, chiefly by Goodrich (1914, 1916) and by Walton (1924). Two of these are X_1X_2 in the male, three others are $X_1X_2X_3X_4X_5$, $X_1X_2X_3X_4X_5X_6$ and $X_1X_2X_3X_4X_5X_6X_7X_8Y$ respectively. Unfortunately the chromosomes are not at all favourable for analysis, since they are small and all about the same size. It is consequently not possible to speculate as to how these multiple sex chromosomes have arisen. The only hypothesis that seems justifiable is that the more complex mechanisms have arisen from simpler ones in several stages. The nature of the meiotic mechanism is also not clear, although it appears likely that some kind of determinate disjunction is involved.

XI. SUMMARY

1. A multiple sex-chromosome mechanism can be defined as one which includes more than one kind of X or one kind of Y . In the Metazoa multiple sex chromosomes occur sporadically in a number of groups.

2. In most cases of multiple sex chromosomes there are several non-homologous, or only partly homologous X 's (X_1, X_2, X_3, \dots) but only one Y . A few species are known in which there are multiple X 's but no Y . Very few animals are known which have a multiple Y .

3. The only groups in which a large number of species all have multiple sex chromosomes are the Nematoda, Reduviidae, Mantoidea and Spiders; in other groups "multiple" mechanisms exist in single species or genera and are hence of fairly recent origin, having arisen in the course of evolution from XY and XO mechanisms.

4. In the first three groups mentioned above XY or XO species occur in addition to those with "multiple" mechanisms; but in the spiders the whole group appear to possess a "multiple" mechanism, which must consequently be of considerable antiquity.

5. In the Nematoda and Reduviidae, where "multiple" systems of different degrees of complexity occur, the more complex ones have probably arisen from simpler ones in the following sort of way:

$$XY \rightarrow X_1X_2Y \rightarrow X_1X_2X_3Y \dots \text{ and } XO \rightarrow X_1X_2 \rightarrow X_1X_2X_3 \dots$$

6. There appear to be two main ways in which multiple mechanisms

can arise: (a) by the inclusion of autosomes or parts of autosomes in the sex-determining mechanism as a result of translocation, (b) by the reduplication of parts of the original sex chromosomes. The first of these modes of origin seems to be found in $XO:XX$ organisms and in groups where the sex chromosomes undergo pairing in the heterogametic sex, the second in those groups where pairing and chiasma-formation between the sex chromosomes has been replaced by a mechanism of "determinate disjunction" in the heterogametic sex.

7. Most of the more complicated types of "multiple" mechanism seem to have arisen by the second method. The first method does not appear to have led to anything more complex than an X_1X_2Y mechanism.

8. A classification of "multiple" mechanism solely according to the number of X 's and Y 's present is superficial because it gives no information about the evolutionary origin of the different types. A detailed analysis and, if possible, a careful comparison with related XY or XO species is needed in each case.

ADDENDUM

Since the foregoing was written a number of papers have been published which deal with multiple sex chromosome mechanisms. In addition a few earlier papers were overlooked when the article was prepared.

(1) *Drosophila virilis americana* (Hughes, 1939) is an XY_1Y_2 subspecies of *D. virilis* and has a chromosome set which is essentially identical with one of the two hypothetical conditions predicted in Fig. 6. It is not, however, directly ancestral to the *pseudoobscura* condition, since the chromosome arm which has been translocated to the X is " E " and " D ", as in *pseudoobscura*.

(2) In the Lygaeidae (Heteroptera) von Pfaler (1937) has described an X_1X_2Y condition in *Eremocoris erraticus* Fab.; the Y is the largest of the three sex chromosomes. In *Lygaeus equestris* L. he found two kinds of males, XY and $XY Y$ (he apparently believed the latter to be XY_1Y_2 —i.e. that their Y 's were not-homologous—but it seems more probable from his figures that the Y 's were homologous).

(3) In *Lepisma domestica* (Thysanura) Perrot (1933) described an $X_1X_2:X_1X_1X_2X_2$ mechanism, but his figures do not permit one to speculate as to its exact nature and origin, particularly since no related species of Thysanura has been studied cytologically.

(4) Darlington (1939) has further investigated the case of *Cimex lectularius*. He also studied *C. columbarius* which is an X_1X_2Y species.

REFERENCES

- ASANA, J. J. (1934). "Studies on the chromosomes of Indian Orthoptera. IV. The idiochromosomes of *Hierodula* sp." *Curr. Sci.* **2**, 244-5.
- DARLINGTON, C. D. (1934). "Anomalous chromosome pairing in the male *Drosophila pseudoobscura*." *Genetics*, **19**, 95-118.
- (1937). *Recent Advances in Cytology*, 2nd ed. London: Churchill.
- (1939). *The Evolution of Genetic Systems*. Camb. Univ. Press.
- (1939). "The genetical and mechanical properties of the sex chromosomes. V. *Cimex* and the Heteroptera." *J. Genet.* **39**, 101-37.
- DOBZHANSKY, TH. (1935). "*Drosophila miranda*, a new species." *Genetics*, **20**, 377-91.
- DOBZHANSKY, TH. & TAN, C. C. (1936a). "A comparative study of the chromosome structure in two related species, *Drosophila pseudoobscura* and *D. miranda*." *Amer. Nat.* **70**, 47-8.
- (1936b). "Studies on hybrid sterility. III. A comparison of the gene arrangement in two species, *Drosophila pseudoobscura* and *D. miranda*." *Z. indukt. Abstamm.- u. VererbLehre*, **72**, 88-114.
- GOLDSMITH, W. M. (1916). "The relation of the true nucleolus to the linin network in the growth period of *Pselliodes cinctus*." *Biol. Bull. Wood's Hole*, **31**.
- (1919). "A comparative study of the chromosomes of the tiger beetles (Cicindelidae)." *J. Morph.* **32**, 437-87.
- GOODRICH, H. B. (1914). "The maturation divisions in *Ascaris incurva*." *Biol. Bull. Wood's Hole*, **27**, 147-50.
- (1916). "The germ cells in *Ascaris incurva*." *J. exp. Zool.* **21**, 61-100.
- HARD, W. L. (1939). "The spermatogenesis of the lycosid spider, *Schizocosa crassipes* (Walckenaer)." *J. Morph.* **65**, 121-54.
- HONDA, H. (1921). "Spermatogenesis of Aphids; the fate of the smaller secondary spermatocytes." *Biol. Bull. Wood's Hole*, **40**, 349-69.
- HUGHES, R. D. (1939). "The chromosomes in the hybrid between *Drosophila virilis virilis* and *Drosophila virilis americana* Spencer." *Genetics*, **24**, 99.
- JUNKER, H. (1923). "Cytologische Untersuchungen an den Geschlechtsorganen der halbzwittrigen Steinfliege *Perla marginata* (Panzer)." *Arch. Zellforsch.* **17**, 185-259.
- KING, R. L. (1931). "Chromosomes of three species of Mantidae." *J. Morph.* **52**, 523-33.
- KING, R. L. & BEAMS, H. W. (1938). "The multiple chromosomes of *Paratylotropidia brunneri* Scudder (Orthoptera: Acrididae)." *J. Morph.* **63**, 289-300.
- LINDNER, E. (1914). "Über die Spermatogenese von *Schistosomum haematobium* Bilh. (*Bilharzia haematobia* Colb.) mit besonderer Berücksichtigung der Geschlechtschromosomen." *Arch. Zellforsch.* **12**, 516-38.
- MATHER, K. & STONE, L. H. A. (1933). "The effect of X-radiation upon somatic chromosomes." *J. Genet.* **28**, 1-24.
- MATTHEY, R. (1931). "Chromosomes de Reptiles Sauriens, Ophidiens et Cheloniens. L'Évolution de la formule chromosomiale chez les Sauriens." *Rev. suisse Zool.* **38**, 117-86.
- MONTGOMERY, T. H. (1901). "A study of the chromosomes of germ cells of Metazoa." *Trans. Amer. phil. Soc.* **20**, 154-236.

- MONTGOMERY, T. H. (1906). "Chromosomes in the spermatogenesis of the Hemiptera Heteroptera." *Trans. Amer. phil. Soc.* **21**.
- MORGAN, L. V. (1938). "Origin of attached-X chromosomes in *Drosophila melanogaster* and the occurrence of non-disjunction of X's in the male." *Amer. Nat.* **72**, 434-46.
- MORGAN, T. H. (1906). "The male and female eggs of the Phylloxerans of the hickories." *Biol. Bull. Wood's Hole*, **10**, 201-6.
- (1908). "The production of two kinds of spermatozoa in Phylloxerans: functional 'female-producing' and rudimentary spermatozoa." *Proc. Soc. Exp. Biol., N.Y.*, **5**.
- (1909 a). "Sex determination and parthenogenesis in Phylloxerans and Aphids." *Science*, **29**, 234-7.
- (1909 b). "A biological and cytological study of sex determination in Phylloxerans and Aphids." *J. exp. Zool.* **7**.
- (1912). "The elimination of the sex chromosomes from the male producing eggs of Phylloxerans." *J. exp. Zool.* **12**, 379-98.
- (1915). "The predetermination of sex in Phylloxerans and Aphids." *J. exp. Zool.* **19**, 285-321.
- MORGAN, W. P. (1928). "A comparative study of spermatogenesis of five species of earwigs." *J. Morph.* **46**, 241-73.
- MULLER, H. J. (1940). Article in *The New Systematics*. Oxford Univ. Press: edited by J. S. Huxley.
- MCCLEUNG, C. E. (1917). "The multiple chromosomes of *Hesperotettix* and *Mermiria*." *J. Morph.* **29**, 519-605.
- McKNIGHT, R. H. (1939). "The sex-determining mechanism of *Drosophila miranda*." *Genetics*, **24**, 180-201.
- NAKAHARA, W. (1919). "A study on the chromosomes in the spermatogenesis of the stone-fly *Perla immarginata* Say." *J. Morph.* **32**, 509-29.
- NAVILLE, A. & DE BEAUMONT, J. (1933). "Recherches sur les chromosomes des Névroptères." *Arch. Anat. Micr.* **29**, 199-243.
- (1936). "Recherches sur les chromosomes des Névroptères. Deuxième partie." *Arch. Anat. Micr.* **32**, 272-302.
- NAWASCHIN, M. (1932). "The dislocation hypothesis of evolution of chromosome numbers." *Z. indukt. Abstamm.- u. VererbLehre*, **63**, 224-31.
- NONIDEZ, J. F. (1914). "Los cromosomas en la espermatogenesis del *Blaps lusitanica*." *Trab. Mus. Cienc. nat., Madr.*, Ser. Zool., **18**.
- (1915). "Estudios sobre las celulas sexuales. I. Los cromosomas goniales y las mitosis de maduracion en *Blaps lusitanica* y *B. walshi*." *Mem. Soc. esp. Hist. nat.* **10**.
- (1920). "The meiotic phenomena in the spermatogenesis of *Blaps*, with special reference to the X complex." *J. Morph.* **34**, 69-117.
- OGUMA, K. (1921). "The idiochromosomes of the mantis." *J. Coll. Agric. Sapporo*, **10**, 1-27.
- PAINTER, T. S. (1914). "Spermatogenesis in spiders. I." *Zool. Jb.* **38**, 509-76.
- (1921). "Studies in Reptilian spermatogenesis. I. The spermatogenesis of lizards." *J. exp. Zool.* **34**, 281-327.
- PAYNE, F. (1908). "On the sexual difference of the chromosome groups in *Galgulus oculus*." *Biol. Bull. Wood's Hole*, **14**, 297-303.

- PAYNE, F. (1909). "Some new types of chromosome distribution and their relation to sex." *Biol. Bull. Wood's Hole*, **16**, 119-66.
- (1910). "The chromosomes of *Acholla multispinosa*." *Biol. Bull. Wood's Hole*, **18**, 174-9.
- (1912 a). "A further study of the chromosomes of the Reduviidae. II. The nucleolus in the young oöcytes and the origin of the ova in *Gelastocoris*." *J. Morph.* **23**, 331-47.
- (1912 b). "The chromosomes of *Gryllotalpa borealis* Burm." *Arch. Zellforsch.* **9**, 141-8.
- (1916). "A study of the germ cells of *Gryllotalpa borealis* and *G. vulgaris*." *J. Morph.* **28**, 287-327.
- PERROT, J. L. (1933). "La spermatogénèse et l'ovogénèse de *Lepisma (Thermobia) domestica*. Hétéropycnose dans un sexe homogamétique." *Z. Zellforsch.* **18**, 573-92.
- VON PFALER, E. (1937). "Das Verhalten der Idiochromosomen zweier Lygaeiden." *Acta. Soc. Fauna Flora Fennica* **60**, 177-89.
- SCHRADER, F. (1928). *Die Geschlechtschromosomen*. Berlin: Borntraeger.
- SEILER, J. (1925). "Ergebnisse aus Kreuzungen von Schmetterlingsrassen mit verschiedener Chromosomenzahl." *Arch. Klauss-Stift. VererbForsch.* **1**, 63-117.
- SHINJI, O. (1931). "The evolutionary significance of the chromosomes of the Aphididae." *J. Morph.* **51**, 373-433.
- SLACK, H. D. (1939 a). "The chromosomes of *Cimex*." *Nature, Lond.*, **143**, 78.
- (1939 b). "Structural hybridity in *Cimex* L." *Chromosoma*, **1**, 104-18.
- (1939 c). Unpublished work.
- STEOPOE, I. (1925). "La spermatogénèse chez la *Nepa cinerea*." *C.R. Soc. Biol., Paris*, **92**, 1476-8.
- STEVENS, N. M. (1906). "Studies in spermatogenesis with special reference to the accessory chromosome." *Publ. Carneg. Instn.*, no. 36.
- (1909). "Further studies on the chromosomes of the Coleoptera." *J. exp. Zool.* **6**, 101-13.
- SUGIYAMA, M. (1933). "Behaviour of the sex chromosomes in the spermatogenesis of Japanese earwig, *Anisolabis marginalis*." *J. Fac. Sci. Imp. Univ. Tokyo. Ser. iv.*
- WALTON, A. C. (1924). "Studies on nematode gametogenesis." *Z. Zell. Gewebe.* **1**.
- WHITE, M. J. D. (1933). "Tetraploid spermatocytes in a locust, *Schistocerca gregaria*." *Cytologia, Tokyo*, **5**, 135-9.
- (1936). "The chromosome cycle of *Ascaris megalocephala*." *Nature, Lond.*, **137**, 783.
- (1938). "A new and anomalous type of meiosis in a mantid, *Callimantis antillarum* Saussure." *Proc. roy. Soc. B*, **125**, 516-23.
- WILSON, E. B. (1909 a). "Studies on chromosomes. IV. The 'accessory' chromosome in *Syromastes* and *Pyrrhocoris* with a comparative review of the types of sexual difference of the chromosome groups." *J. exp. Zool.* **6**, 69-99.
- (1909 b). "The female chromosome groups in *Syromastes* and *Pyrrhocoris*." *Biol. Bull. Wood's Hole*, **16**, 199-204.
- (1925). *The Cell in Development and Heredity*. New York: Macmillan.
- (1911). "The sex chromosomes." *Arch. mikr. Anat.* **77**, 249-71.

THE GENETICS OF *VERBENA*. I

BY G. H. BEALE

John Innes Horticultural Institution, Merton

(With Plates XIII-XV)

CONTENTS

	PAGE
1. Introduction	337
2. Origin and development of the present garden strains	338
3. Material and methods	339
4. Inheritance of individual characters	339
5. Discussion:	
(i) Ratios	352
(ii) Modifying factors	353
(iii) Dominance	354
(iv) Linkage	356
6. Summary	357
References	358
Explanation of Plates XIII-XV	358

1. INTRODUCTION

MOST organisms which have been investigated genetically owe their variability to gene mutation within a single species. The garden *Verbena* is exceptional in this respect, in that a great part of its variability can be traced back to differences between three or four original wild species which were crossed together by horticulturists. It is important to know whether there is any difference between the inheritance of interspecific and intraspecific differences, but interspecific differences are notoriously difficult to study genetically because of such complications as sterility of the hybrids and the absence of clear segregation of the various characters.

In *Verbena*, however, some hundred years have elapsed since the initial crosses were made, and selection has led to the production of comparatively fertile and true-breeding strains. Here there is an opportunity for studying the inheritance of interspecific and intraspecific differences in the same plant. There is the added advantage with *Verbena* that the diploid chromosome number is always 10, so that complications due to polyploidy do not arise (as, for example, they do in the New World cottons). Moreover, the small number of chromosomes makes the determination of linkages less laborious.

2. ORIGIN AND DEVELOPMENT OF THE PRESENT GARDEN STRAINS

The genus *Verbena* is divided into two sections, *Verbenacea* and *Glandularia* (de Candolle, 1847). All the forms considered here belong to the *Nobiles*, one of the five groups of the *Verbenacea* section. All members of the *Glandularia* section and of the *Nobiles* group of the *Verbenacea* section (so far determined) have the basic number $n=5$. (For lists of chromosome numbers see Dermen (1936), and Noack (1937). The following additional numbers are given here: *V. peruviana* (*chamaedrifolia*) $2n=10$; *V. radicans* $2n=10$; *V. tenera* $2n=30$.)

On horticultural evidence (see Bailey, 1902), it is considered that the garden *Verbena*, "*V. hybrida*" (Voss.) has been derived from crosses between the following four species:

- (1) *V. peruviana* (L.) Britton (= *V. chamaedrifolia* Juss.).
- (2) *V. phlogiflora* Cham. (= *V. Tweediana* Niven).
- (3) *V. incisa* Hook.
- (4) *V. platensis* Spreng. (= *V. teucrioides* Gill & Hook.).

Brief notes regarding these species are given in Table I. No attempt is made to give rigorous taxonomic descriptions, but the distinguishing characters are emphasized, especially those which segregate in the modern forms.

Of the four species *V. incisa* is probably a variety of *V. phlogiflora*, but the others are regarded as distinct. The modern cultivated forms have characters from all the wild prototypes: e.g. the colour of the flowers may be bright scarlet, scarlet-magenta, or very pale; the inflorescence may be an extremely flat corymb, a very long spike, or intermediate; the eye varies in size, and may or may not have a coloured inner rim.

There is little doubt that crossing has been attempted by horticulturists. Lecocq (1852) reports having crossed *V. incisa*, *V. "teucrioides"* (= *V. platensis*) and *V. "Melindres"* (= *V. peruviana*), giving an account of a technique for emasculation and pollination much the same as that used in this work.

Some of the variations of the modern garden strains appear to have arisen as mutations after the original crosses were made, e.g.:

- (1) Pure white flowers.
- (2) Yellow flowers (said to have appeared in 1896).
- (3) Deep bluish purple flowers (first appeared in 1854).
- (4) Compact habit (arose in 1870 at Erfurt).
- (5) Striped or flaked flowers (known already in 1849).

Further, as a result of inbreeding, a number of semi-lethal types have appeared in the experimental cultures.

From the above account it is possible to gain an idea of the extent to which the present variability is due to the original species differences, and to subsequent mutation.

3. MATERIAL AND METHODS

Seeds of *V. platensis* were kindly sent by Dr K. L. Noack (Berlin) and of other species by Dr H. N. Moldenke (New York) and Dr Haig Dermen (Harvard). All the garden hybrid material was obtained from commercial sources. The varieties, where known, from which the various factors have been obtained are named in the descriptions of the inheritance of the individual factors.

The germination of the seed was rarely above 50 %, the most favourable results being obtained by soaking the seed in water for 24 hr. immediately before sowing. Self-fertilization occurs naturally except in a few forms in which the stigmas project out of the corolla tube. Plants required for selfing were isolated in muslin-covered cages. Paper bags proved unsatisfactory. Emasculation was carried out by removing the entire corolla at an early stage (i.e. when still folded up); the stamens, being epipetalous, were removed at the same time. Pollination was usually effected 2 days after emasculation. With hot dry weather the seed took about a month to ripen, but it was rare to obtain the maximum number of seeds (four) per flower. Cuttings of parent plants were taken and grown in the succeeding years for comparison with their progeny.

The heterogeneity in segregation of a factor in different families was determined by the Brandt and Snedecor formula (quoted by Mather, 1938), i.e.

$$\chi^2 = \frac{n_t^2}{a_{1t}a_{2t}} \left[S \left(\frac{a_1^2}{n} \right) - \frac{a_{1t}^2}{n_t} \right]$$

Families in which the smallest expected class contained less than eight plants were grouped together, where possible taking closely related families in a group.

4. THE INHERITANCE OF INDIVIDUAL CHARACTERS

From the foregoing account of the origin of the garden strains it is obviously not possible to refer to a particular wild type. For convenience, however, we shall consider the standard type to be like *V. incisa*, as described in Table I, but with a deep purple flower colour. All other forms will be taken as deviations from this type.

TABLE I

Species	Chromo- some no. $2n=10$	Habit	Inflorescence	Flower colour	Eye of flower	Introduced	Figure
(1) <i>V. peruviana</i>	—	Very prostrate	Flat corymb	Bright scarlet	Very small, yellow with dark inner rim	ca. 1826 from Argentina	As <i>V. chamaedrifolia</i> in <i>Bot. Mag.</i> 1833, pl. 3333
(2) <i>V. phlogiflora</i>	—	Upright	Spike longer than (1)	Scarlet-magenta	Medium size, yellow	1834, from Rio Grande do Sul (Brazil), etc.	As <i>V. Tweediana</i> in <i>Bot. Mag.</i> 1835, pl. 3541
(3) <i>V. incisa</i>	—	Upright, leaves more deeply lobed than (2)	Corymb broad and depressed (as (1))	Scarlet-magenta, fading	Medium size, yellow	1836, Santa Fé (Argentina), etc.	<i>Bot. Mag.</i> 1837, pl. 3628
(4) <i>V. platensis</i>	$2n=10$	Upright; more erect than (1)-(3)	Spike 6-9 in., much longer than (1)-(3)	When opening, flowers white; later pale pink	Medium size, yellow, with conspicuous dark inner rim	1837, from Buenos Ayres	As <i>V. teucrioides</i> in Paxton, <i>Mag. Bot.</i> 1838, 5, 243

TABLE II

Factors	Dominants*	Recessives	No. of families	Deviation χ^2	D.F.	P	Heterogeneity χ^2	D.F.	P
A-a	204	23	10	26.76	1	<0.01	3.25	5	0.7 -0.5
D-d	1606	590	23	4.08	1	0.05-0.02	30.22	17	0.05-0.02
M-m	2136	844	22	17.54	1	<0.01	77.64	16	<0.01
M ^d -m	418	145	2	0.17	1	0.7 -0.5	0.05	1	0.9 -0.8
P-p	1820	542	22	5.31	1	0.05-0.02	16.15	16	0.7 -0.5
P ^d -p	1254	398	8	0.73	1	0.5 -0.3	10.88	6	0.2 -0.1
U-u	1431	282	11	66.59	1	<0.01	11.80	10	0.3 -0.2
U ^d -u	985	456	17	33.93	1	<0.01	31.17	13	<0.01
E-e	1591	770	21	72.99	1	<0.01	108.37	15	Very small
R-r	2100	877	20	31.57	1	<0.01	71.35	19	<0.01
C-c	1146	124	6	157.24	1	Very small	50.54	4	<0.01
I-i	70	14	3	3.11	1	0.1 -0.05	1.61	1	0.3 -0.2
S-s	833	708	13	360.52	1	Very small	63.41	9	<0.01
W-w	1045	90	7	176.40	1	Very small	90.75	4	Very small
Z-z	494	339	7	109.46	1	Very small	110.89	6	Very small
G-g	63	21	2	0	1	1.0	0.31	1	0.7 -0.5
N-n	586	200	4	0.08	1	0.8 -0.7	16.96	3	<0.01

* Where dominance is incomplete, the figures in the "dominant" column refer to the allelomorphs represented by the capital letter.

Data on the segregation of seventeen pairs of factors are summarized in Table II. The families are all of the F_2 type (i.e. selfed heterozygotes). Since many factors give heterogeneous results, the totals given can only be taken as a rough indication of the actual segregations. The detailed figures are too bulky to be included, but are available for reference.

(a) *Flower colour factors*

(i) *Coloured-white flowers (A-a).*

Pure white flowers are completely recessive to coloured (i.e. by anthocyanin) and give in F_2 a significant and consistent deficit of the recessive type. Mention may be made here of the flaking whites, though it is intended to give a more detailed account in a separate paper. The flakes, which may be of any colour, vary greatly in size and number. On selfing a flaked plant, occasional fully coloured plants occur in the progeny, and on selfing such a fully coloured plant approximately three full to one flaked are obtained (actual numbers 168 : 68). Self-fertilization of a flake obtained from some mixed seed yielded three flakes to one non-flaking white (actual numbers 61 : 21). Hence in flaked plants there is frequent mutation from recessive white to dominant coloured. The genetical relation between flaking and non-flaking whites, and between the various grades of flaking, has not been fully determined.

(Source of non-flaking whites: "hybrida alba", "Snowball". Source of flaking whites: "hybrida striata".)

(ii) *White-yellow flowers (Ye-ye and modifiers).*

If yellow flowers are crossed with white, the F_1 is white and in the F_2 a range of colours is obtained varying between the two grandparents; a rough classification gave 25 white, 8 pale cream and 1 yellow. Yellow flowers therefore contain the allelomorph *a*, and probably two other factors, one of which (*ye*) is recessive. The effect of *ye* on *A* types is not known.

(Source of *ye*: "lutea improved".)

(iii) *Full-pale-dilute flowers (DD-Dd-dd).*

"Dilute" flowers are white when first opening, but later on develop a certain amount of anthocyanin. The type is incompletely recessive, the heterozygote being paler and bluer than the standard (see Pl. XIII). In Table II *DD* and *Dd* types were grouped together, since most families were recorded before it was realized that the heterozygotes could be distinguished. More detailed data are presented in Table III. The species

V. platensis has a colour exactly like that of the *dd* plants, and on crossing the two together an F_1 was obtained having dilute flowers. Therefore the species *V. platensis* carries the allelomorph *d*, and is probably the source of this allelomorph in the garden hybrids.

The F_1 between dilute and white (*a*) is coloured, showing that the two factors are not allelomorphic.

(Source of dilute flowers: forms extracted from "hybrida purpurea".)

(iv) *Dominant maroon- purple- recessive maroon (M^d-M-m)*.

(v) *Dominant scarlet-magenta- purple- recessive scarlet-magenta (P^d-P-p)*.

These types may conveniently be considered together. If a purple form (derived from the variety "hybrida purpurea") is crossed with a scarlet ("hybrida Fireball") the hybrid is purple, and in the F_2 appear the following four colour types: purple, maroon, scarlet-magenta and scarlet

TABLE III
Segregation of dilute

Type of family F_2	DD	Dd	dd	No. of fami- lies	Devia- tion χ^2	P for devia- tion of total from expected ratio	Hetero- geneity χ^2	P for hetero- geneity
	57	155	72	8	3.96	0.2-0.1	18.56	0.1-0.05
Backcross:								
♀ <i>Dd</i> × ♂ <i>DD</i>	46	52	—	6	0.39	0.7-0.5	—	—
♀ <i>DD</i> × ♂ <i>Dd</i>	6	3	—	1	—	—	—	—
♀ <i>Dd</i> × ♂ <i>dd</i>	—	8	13	1	—	—	—	—

(see Pl. XIII), in the ratio of 9 : 3 : 3 : 1. If the same purple is crossed with another strain of scarlet ("Miss Willmott"), the F_1 is not purple but scarlet, and in the F_2 the four colour types (purple, maroon, scarlet-magenta and scarlet) appear in the ratio of 1 : 3 : 3 : 9. Crossing the two strains of scarlets (henceforward called "recessive" and "dominant" scarlets) produces in F_1 and F_2 nothing but scarlet-flowered plants. Data supporting these facts are presented in Table IV.

The dominant and recessive scarlets are visibly indistinguishable, and each of the four colours obtained from the cross purple × dominant scarlet is identical with one obtained from the cross purple × recessive scarlet. The pigments are the same no matter which strain of scarlet has been used.

From Table IV it is evident that purple differs from recessive scarlet by two dominant factors and from dominant scarlet by two recessive factors. The genetic relationship between the dominant and recessive scarlets can also be determined from the data in Table IV. For simplicity only one character will be considered at first, i.e. the difference purple-

maroon. There are three possible schemes: (1) recessive maroon differs from purple by a single factor **m**, and dominant maroon has a dominant inhibitor **I**. The dominant maroon may contain (a) **M** or (b) **m**. (2) Both dominant and recessive maroons contain a factor **m**, the difference between the two being that the latter contains a dominance modifier, which may itself be (a) dominant or (b) recessive. (3) There is a series of three allelomorphs, **M^d**, **M**, **m**, corresponding respectively to dominant maroon, purple and recessive maroon.

In the F_2 's derived from the three types of cross described in Table IV, various ratios are expected according to which of the three schemes postulated above is the correct one. In Table V the various expected

TABLE IV
Segregation of the colours purple, maroon, scarlet-magenta and scarlet

Parents	...	F_1	F_2				χ^2	D.F.	P
			Purple	Maroon	Scarlet-magenta	Scarlet			
Purple \times recessive scarlet		Purple	746	256	227	85	[to 9:3:3:1] 2.07 (8 families)	3	0.7-0.5
Purple \times dominant scarlet		Scarlet	35	89	97	294	[to 1:3:3:9] 0.88 (1 family)	3	0.9-0.8
Recessive scarlet \times dominant scarlet		Scarlet	—	—	—	719	— (4 families)	—	—

ratios are set out, together with the values of χ^2 calculated from deviations of the obtained results from expectation. It will be seen that the only scheme which gives throughout no significant χ^2 is the third, i.e. that in which there is a series of multiple allelomorphs. Consequently this is taken to be the true system. A similar analysis of the data was made for the difference purple—scarlet-magenta, and a similar result obtained, i.e. a series of multiple allelomorphs **P^d**, **P**, **p** corresponding to the characters dominant scarlet-magenta, purple, recessive scarlet-magenta respectively. The **P** series of allelomorphs shows no linkage with the **M** series.

One further possibility must be considered. If (in Table V) **m** is closely linked with **i** or with **d**, the results obtained would be like those expected under the multiple allelomorph hypothesis. A limit can be set to the looseness of such linkages, and if it is found that, with the figures already obtained, linkage must be very close, there is no reason why the multiple allelomorph hypothesis need be abandoned.

Suppose **m** and **d** are linked and have a cross-over value *p*. Then in

F_2 from dominant maroon (**mD**) × purple (**Md**) the expected ratio of purple to maroon is

$$\frac{1}{4}n(1+2p-2p^2) : \frac{1}{4}n(3-2p+2p^2),$$

where n = total number of plants. (If $p=0$, the ratio is 1 : 3; if $p=\frac{1}{2}$, the ratio is 6 : 10.) The standard error for the expected numbers of purples and maroons is

$$\sigma = \sqrt{\frac{(1+2p-2p^2)(3-2p+2p^2)n}{16}}.$$

The results obtained do not differ significantly from a 1 : 3 ratio (see

TABLE V

Obtained	→	F_2 from purple × recessive maroon		F_2 from purple × dominant maroon		F_2 dominant maroon × recessive maroon	
		Purple 973	Maroon 341	Purple 132	Maroon 383	Purple 0	Maroon 719
(1a) (inhibitor):							
Dominant maroon MI		3 : 1		1 : 3		3 : 13	
Purple Mi		$\chi^2=0.63$		$\chi^2=0.11$		$\chi^2=165$	
Recessive maroon mi		$P=0.9-0.8$		$P=0.95-0.90$		$P=\text{Very small}$	
(1b) (inhibitor):							
Dominant maroon mI		3 : 1		3 : 13		0 : all	
Purple Mi		$\chi^2=0.63$		$\chi^2=16.01$		$\chi^2=0$	
Recessive maroon mi		$P=0.9-0.8$		$P=<0.01$			
(2a) (dominance modifier):							
Dominant maroon mD		3 : 1		6 : 10		0 : all	
Purple Md		$\chi^2=0.63$		$\chi^2=30.4$		$\chi^2=0$	
Recessive maroon md		$P=0.9-0.8$		$P=<0.01$			
(2b) (dominance modifier):							
Dominant maroon md		10 : 6		1 : 3		0 : all	
Purple Md		$\chi^2=74.77$		$\chi^2=0.11$		$\chi^2=0$	
Recessive maroon mD		$P=<0.01$		$P=0.95-0.90$			
(3) (multiple allelomorphs):							
Dominant maroon M^d		3 : 1		1 : 3		0 : all	
Purple M		$\chi^2=0.63$		$\chi^2=0.11$		$\chi^2=0$	
Recessive maroon m		$P=0.9-0.8$		$P=0.95-0.90$			

Table V, middle column). Assuming linkage, the deviations from expectation on a 1 : 3 are

$$\frac{1}{4}n[3-(3-2p+2p^2)] = \frac{1}{2}np(1-p).$$

Putting this equal to 2σ , we have

$$\frac{1}{2}np(1-p) = 2 \sqrt{\frac{(1+2p-2p^2)(3-2p+2p^2)n}{16}},$$

$$\therefore p^4(n+4) - 2p^3(n+4) + p^2(n+8) - 4p - 3 = 0.$$

From Table V, $n=132+383=515$,

$$\therefore 519p^4 - 1038p^3 + 523p^2 - 4p - 3 = 0,$$

$$\therefore p=0.087,$$

therefore, with the number of plants obtained, crossing-over would be detected with reasonable certainty if it were greater than 8.7 %. This applies to both **m** and **p** and their respective modifiers.

Similarly, if **m** and **i** are linked, the expectations of purple and maroon in F_2 from **mI** × **Mi** are

$$\frac{1}{4}n(1-p^2) \quad \text{and} \quad \frac{1}{4}n(3+p^2)$$

respectively, and

$$\sigma = \sqrt{\frac{(3+p^2)(1-p^2)n}{16}}.$$

Deviations from expectation on 1 : 3 are

$$\frac{1}{4}np^2.$$

Putting this equal to 2σ we obtain

$$p^4(n^2 + 4n) + p^2(8n) - 12n = 0.$$

Then, from Table V, $n = 515$. Therefore $p = 0.38$, i.e. crossing-over between the factors **m** and **p** and their respective inhibitors would be detected if it were greater than 38 %.

There is some evidence that tight linkages do occur in *Verbena* (see below under the factors **c** and **u**) and in particular near the locus of **m**. Nevertheless, it is unlikely that both **m** and **p** would be closely linked with their respective modifiers or inhibitors; assuming that the genes are uniformly distributed in all five chromosomes, the probability would be considerably less than one in 25, since **p** and **m** are independent. Non-random distribution of genes would increase this probability, but on the other hand recombination by crossing-over would decrease it.)

Further tests will be necessary to confirm the "multiple allelomorph" hypothesis, since evidence which is based on agreement or disagreement with ratios is not wholly conclusive in *Verbena*, where so often deviations from the expected ratios have occurred, presumably owing to decreased viability of one of the genotypes, or to linkage with lethal factors.

There are undoubtedly more factors controlling the four colour types described above, as will be seen from the following. In crossing the dominant scarlet with a new stock of purple (from the variety "Violet Bouquet") the following numbers were obtained in F_2 : scarlet, 388; scarlet-magenta, 26; maroon 145, and purple 10. Assuming the same genetical constitutions as before, these results would give **P^d** : **P** equal to 414 : 155 (3 : 1 as expected), but **M^d** : **M** equal to 534 : 35. The latter is a very close approximation to a 15 : 1 ratio ($\chi^2 = 0.01$), indicating that the difference between dominant maroon and purple is here bifactorial.

Moreover, in some strains dominance of scarlet-magenta over purple is incomplete, the heterozygote being approximately intermediate in appearance between the two homozygotes. In Table VI are collected data showing this. Each of these families except 3/35 was obtained by selfing an "intermediate" plant in one of the previous families. If a scarlet-magenta or purple plant was self-fertilized, the progeny obtained were all like the parent.

Three purple plants from one of these families were crossed with dominant scarlet (P^dM^d), and the F_1 was found to be scarlet (not intermediate). Two scarlet-magenta plants from one of the families in Table VI were crossed with the stock purple (PM), and the hybrid was intermediate in colour. Therefore, in the families with intermediate dominance the purple is similar to that already described, but the

TABLE VI

*F₂ families showing intermediate dominance of purple
and scarlet-magenta*

Ref. no.	Scarlet-magenta	Intermediate	Purple
3/35	5	22	12
15/36	4	18	7
75/38	8	7	4
80/38	14	19	9
Total	31	66	32

scarlet-magenta differs from both dominant and recessive scarlet-magentas. Presumably the intermediate scarlet-magenta contains another allelomorph in the series P^d-P-p , distinguished from P^d and p only by its dominance potency.

The inheritance of the four colour types is evidently very complicated, and it will require further elucidation. The above described results have only been obtained by utilizing chemical tests on the anthocyanin pigments. As will be described in a second paper (Beale & Scott-Moncrieff), various simple chemical differences are associated with the changes in colour of the flowers, and it would not always have been possible to separate the genotypes without a knowledge of such differences, since very similar changes in colour may be brought about by several distinct chemical changes. Especially where dominance is incomplete, the chemical identification of the pigments has been of the greatest assistance in classifying the phenotypes. (Source of material: purple (PM), "hybrida purpurea"; dominant scarlet (P^dM^d), "Miss Willmott"; recessive scarlet (pm), "hybrida Fireball"; recessive maroon (Pm), also extracted from "hybrida purpurea".)

(vi) *Purple-plum.*

The colour described as plum is illustrated in Pl. XIII. The genetical constitution of plum was investigated by a series of crosses, the results of which are collected in Table VII. From the cross (1), plum is seen to differ from dominant scarlet-magenta (P^dM) by a single recessive factor (which will be designated u), although there is a significant deficit of the u type in F_2 . From the cross (2), plum is seen to differ from purple (PM) by two factors, the first (P^d) being dominant and giving a good approximation to a 3 : 1 in F_2 , the second (u) being recessive and giving a significant deficit. From crosses (3) and (4), plum (uP^dM) and plum-purple (uPM) \times dominant scarlet (UP^dM^d), it is evident that u and M are very closely linked (or allelomorph), for no types corresponding to plum appear in the

TABLE VII
Inheritance of plum

Parents	F_1	F_2				
		Scarlet-magenta	Purple Plum	Plum-purple	Scarlet	Maroon
1. Plum $\times P^dM$	Scarlet-magenta	59	—	10	—	—
2. Plum $\times PM$	"	345	108	64	19	—
3. Plum $\times P^dM^d$	Scarlet	—	—	52	—	251
4. Plum-purple $\times P^dM^d$	"	—	—	18	10	141
5. Plum $\times pm$	Pale scarlet-magenta	328	—	109	—	166
		(pale)				

scarlet or maroon (M) classes. This is confirmed by cross (5), plum (uP^dM) \times recessive scarlet, (Upm), since no Um types appear in the F_2 .

The factor u has been kept separate and not made an allelomorph in the M series because, as can be seen from cross (5), plum differs from scarlet by two characters; for one of these, the difference between plum and scarlet-magenta, plum carries the recessive allelomorph; for the other, the difference between scarlet-magenta and recessive scarlet, plum carries the dominant allelomorph. These two characters are also chemically quite distinct. In effect they are so closely linked that in the F_2 an apparent 1 : 2 : 1 is produced.

There is another factor c very closely linked with both m and u (see below), indicating some special arrangement near the locus of m , c and u .

A limit may be put to the maximum possible amount of crossing-over between u and M^d as follows:

Suppose the true crossover value is p . In F_2 from the cross dominant scarlet (UM^d) \times plum (uM), the expected proportions of plum + scarlet to the other types (UM and uM^d) are:

$$\frac{1}{2}n(2-2p+p^2) : \frac{1}{2}n(2p-p^2)$$

and

$$\sigma = \sqrt{\frac{1}{4}\{n(2-2p+p^2)(2p-p^2)\}}.$$

The data obtained (see Table VII) give a ratio of $n : 0$.

Deviation is $\frac{1}{2}n(2p - p^2)$.

Putting this equal to 2σ , we have

$$p^2(n+4) - 2p(4+n) + 8 = 0,$$

$n = 485$, $p = 0.0082$, i.e. crossing-over between **u** and **M**^d must be less than 0.82 %.

(Source of plum flowers: ex F_2 from "Miss Willmott" \times "hybrida purpurea").

(vii) *Size of eye of the flower (E-e and modifiers).*

The term "eye" refers to a pale greenish yellow region in the centre of the flower, not including the coloured or white hairs which lie over the opening of the corolla tube. "Eyeless" (**ee**) is recessive, but the F_2 figures do not give a very good approximation to a 3 : 1, and there is considerable heterogeneity. In some families however, a good 3 : 1 is obtained. The character is at times not easy to record, since **e** types may develop a trace of the yellow eye, and the size of the **E** types varies considerably.

One strain has been obtained having the "eye" extending over practically the whole flower, leaving only a tinge of anthocyanin at the edge. On crossing this with the normal **E** form, the F_1 has an eye intermediate in size between the two parents, and in F_2 a range of types is obtained; the plants were roughly classified into 41 tinged : 185 as F_1 : 341 normal **E**. Evidently several factors are concerned here. The various forms of eye are illustrated in Pl. XV.

V. peruviana has a very small eye, while *V. incisa* and *V. phlogiflora* each have one of medium size. *V. platensis*, on crossing with an **ee** form, produces an F_1 which is practically eyeless. *V. platensis* therefore probably carries the allelomorph **e** even though it cannot be observed in the phenotype on account of the extreme paleness. (The factor **e** is unscorable on **dd** forms.)

(Source of **ee** flowers: "hybrida Fireball". Source of tinged flowers: "Violet Bouquet".)

(viii) **R-r**.

In *V. peruviana* and *V. platensis*, but not in other species, there is a streak of anthocyanin pigment round the inner margin of the eye, and this character may or may not occur in the garden strains (see Pl. XIII). Presence of the streak is dominant to absence, but may not be completely so. The character can sometimes be quite clearly determined and gives good ratios, but more often it fluctuates markedly in intensity, and is

difficult to score. The combined F_2 data in Table II show a very poor agreement with a 3:1, and much heterogeneity. There is, however, one main factor (**R**) concerned.

(Source of **R** flowers: "hybrida purpurea".)

(b) *Structural characters*

(ix) *Normal-compact habit (C-c).*

The compact form (see Pl. XIV) is distinguished from the normal by having shorter internodes and no runners. Compact is always easily distinguishable and completely recessive, but, as can be seen from the F_2 data in Table II, there is a significant excess of the recessive type, and results in different families are heterogeneous. This is presumed to be due to linkage of other factors affecting viability.

There is a remarkably close linkage **c** and **m**, as shown by data in Table VIII.

TABLE VIII

Coupling F_2 data showing linkage of C and M (13 families)

CM	Cm	cM	cm
1851	2	5	760

(Table VIII includes three families derived from the cross **cmUp** × **CMuPd** (compact recessive scarlet × plum) which are shown separately in Table IX.)

TABLE IX

F_2 derived from cmUp × CMuPd (3 families)

Plum (Mu)		Scarlet-magenta (MU)		Scarlet (mU)	
C	c	C	c	C	c
109	0	327	1	1	165

As already noted, there is apparently complete linkage between **M** and **U**. There is, however, rare crossing-over between **C** and **M**, amounting to 0.23 ± 0.13 %, as calculated by the product method for a coupling F_2 . Repulsion data are not yet available.

The gene **c** has once mutated somatically under experimental conditions, when a **c** plant produced a long shoot having flowers of the same colour as the unmutated part. On selfing flowers on the sported part, 4 normal and 3 compact plants were obtained.

Some data on what is presumed to be the same factor as **c** have been published by Emsweller & Blodgett (1937), who obtained a good approximation to a 3:1 in an F_2 and to a 1:1 in a backcross.

(Source of compact plants: "hybrida Fireball".)

(x) *Normal—long spikes (I-i and modifiers).*

“Long spike” is illustrated in Pl. XV. The inflorescence may be as long as 20 cm. and the corolla tubes also are longer than in the normal. Long spike is recessive to normal in F_1 , but in the F_2 only a very small proportion of long spiked types appear, and there is a certain amount of intergrading. A ratio of approximately 15:1 is sometimes obtained for normal to long inflorescences, indicating a bifactorial system. The detailed figures are given in Table X. *Verbena platensis* has a long inflorescence, and this is due to the same genetic factors as that in the

TABLE X
Segregation of normal—long spike in F_2

Ref. no.	Normal	Long spike
11/35	9	5
75/38	17	2
79/38	229	8
80/38	50	6
81/38	453	26
90/39	388	77
Total	1146	124

cultivated strains, for on crossing the two a typical long-spiked type is obtained.

(Source of long spike: ex “hybrida purpurea”.)

(xi) *Normal-stellate flowers.*

This factor affects chiefly the petal lobes, which are bent downwards on a radial axis. The whole plant is reduced in size, bears few flowers and sets little seed. The stellate character is recessive, and there is a significant and consistent deficiency of the recessive type in F_2 , presumably owing to its reduced viability. There is a close linkage between *s* and *p*, as shown by data in Table XI. The two factors are known also in the repulsed

TABLE XI
Linkage of S and P as shown by coupling F_2 's

	PS	Ps	pS	ps
	24	0	0	5
	25	0	0	4
Total	49	0	0	9

condition; consequently there is probably a genuine linkage here, and not merely pleiotropism.

(Source of stellate flowers: ex “hybrida alba”.)

(xii) *Normal-wrinkled petals (W-w)*.

Wrinkled is shown in Pl. XV. Both lobes and tube of the corolla are wrinkled. Since the stigma projects above the anthers, self-fertilization does not occur naturally. Though there is considerable variation in the degree of wrinkling, segregation is always quite clear, and wrinkling is recessive. Nothing approaching a 3 : 1 ratio is obtained, as will be seen from Table II, since there is usually a pronounced excess of the recessive form, giving almost equality of the two types in F_2 . There is however considerable heterogeneity. The existence of the excess of *w* types is difficult to explain; they are somewhat less vigorous than the normal.

(Source of *w* types: ex "hybrida Fireball".)

(xiii) *Normal—bleached dwarf (Z-z)*.

Bleached dwarf plants are very small, and partially chlorotic. The cotyledons and first leaves are green, but later leaves are practically white. It has never flowered at Merton. The type is evidently recessive, but gives very deficient ratios. It may be described as a semi-lethal.

Winge (1937, p. 87) has published data on a factor called "chlorina" which is evidently very similar to the bleached dwarf described here, and a few of this chlorina type flowered. From a selfed heterozygote 333 normal and 115 chlorina plants were obtained, indicating a single-factor difference.

(Source of bleached dwarf: ex "Miss Willmott".)

(xiv) *Normal-strap leaves (G-g and modifiers)*.

The leaf-type "strap" is shown in Pl. XIV. It is very variable; there are forms with very narrow ribbon-like leaves and others almost normal, but strap can always be distinguished if the first pair of leaves is examined. These are narrow and entire, while in the standard type they are broader and serrate. Cotyledons are unaffected, and later leaves in the less extreme forms of strap are completely normal. The more extreme forms fail to flower, or flower only in the second season after sowing.

Strap is completely recessive in F_1 , but in F_2 (see Table II) a large excess of the recessive type appears.

(Source of strap: ex "hybrida purpurea".)

(xv) *Normal-nana (N-n)*.

"Nana" is a form having very small plants (maximum height about 6 in.), leaves curled downwards and somewhat wrinkled and mottled. Usually a few flowers, which are of normal size, are produced, and seed-

setting is good. The factor is a simple recessive, giving good ratios. Its origin is uncertain; it may have arisen at Merton as a mutation.

(xvi) *Normal-small* (**X-x**).

"Small" differs from "nana" in being larger (about 1 ft. high) and quite normal as regards leaf-shape and colour. A characteristic of **x** plants is that they are late flowering. As shown in Table II, there is good agreement with expectation on a monofactorial scheme, but on account of some irregular families there is significant heterogeneity.

(Source of **x**: ex "hybrida purpurea"² or a mutation.)

5. DISCUSSION

(i) *Ratios*

The inheritance of fourteen pairs of factors and two series of three multiple allelomorphs has been described in the previous section. Reference to Table II will show that many of these so-called factors give deviating F_2 ratios and that often different families give different ratios for the same "factor". Admittedly it cannot be assumed that a given difference is inherited in a *simple* Mendelian fashion until good agreement with the expected ratios is consistently obtained. It has however been thought desirable to present the existing data on a number of characters which may later be shown to be inherited in some more complicated manner. Some of these characters have given perfectly simple segregations in certain families, while in other families more irregular results have been obtained. It would obviously be misleading to present only the straightforward figures. But where there are some families showing no deviations from the expected ratios, there is most probably a simple factorial scheme; in the deviating families modifying factors would be present.

The factors, **d**, **m**, **e**, **r**, **c**, **s**, **g**, **n**, **x** have given good 3 : 1 F_2 ratios in one or more families, but the total figures in Table II obscure this, since other, deviating, families have also been included. The anomalous results cannot be due to faulty recording with the factors **d**, **m**, **c**, **s**, **g**, **n**, **x** or **w** as these types can always be distinguished without difficulty from the standard. However, the types **e** and **r** are sometimes difficult to distinguish from their dominant allelomorphs, and the apparent deviations in segregation of these factors may therefore be due to incorrect scoring.

Some factors, e.g. **a**, **u**, **i**, **w**, **z**, have never given normal ratios. All of these except **w** have given a deficit of the recessive type. This may well be explained for **a**, **u** and **z** as being due to decreased viability. Though no

quantitative estimate of viability has been made, it has been observed that the full number of seeds per flower (4) is usually not obtained, and of the seeds which ripen usually less than 50 % germinate. Hence gametic and zygotic elimination, due to decreased viability of a particular genotype, may well take place.

This argument cannot be used to explain the anomalous segregations obtained with the factor **w**, since here the recessive type, though in appearance somewhat weaker, is obtained in F_2 in numbers almost equalling those of the dominant type, no matter whether the recessive allelomorph is transmitted through the male or female side. As regards **i**, it has already been noted that sometimes a 15:1 ratio is obtained for normal to long spike, while at other times a greater proportion of the long spike type occurs. Further investigation is required for these two characters. Considering that there can be only five linkage groups, and that many semi-lethal forms appear in the seed pans but do not survive, it is likely that there are lethal and semi-lethal factors linked with the recorded ones; this would be sufficient to explain the results with **w** and **i**.

The material of *Verbena* is probably exceptional in showing such a large number of deviating and heterogeneous families, but it is rather difficult to make a comparison with other organisms since it has so frequently been the custom to give only figures agreeing with expectation on a simple factorial basis and to omit deviating results.

(ii) *Modifying factors*

A second unusual feature of *Verbena* is the large number of modifying factors. A few genotypes, e.g. **a**, **c**, **u**, **n**, **x**, **z**, are constant in appearance, but many of the others show a certain amount of variation within the type. This is particularly so with **w** and **g** (see Pl. XIV for the latter), where an enormous range of variation occurs within the recessive type, even though it can be quite clearly distinguished from the dominant. Such variation must be due to modifying factors which affect the appearance of **w** or **g** plants, but have no apparent influence on those containing the corresponding dominant allelomorphs. Similarly, there is one main recessive factor distinguishing yellow from white flowers, as previously described, but a considerable range of intermediate colours is obtained in F_2 , indicating the presence of modifiers.

As regards the differences **E-e**, **R-r** and **I-i**, there is considerable variation among both dominant and recessive types. The allelomorphs **M^d**, **M**, **m** and **P^d**, **P**, **p**, which are also rather variable in expression, will be considered below.

In general it appears that modifying factors are unusually plentiful in the garden *Verbena*, though here again one cannot be sure that this is really exceptional, since there is always a tendency to ignore characters showing complex and variable inheritance, and to concentrate on those giving clear monofactorial differences.

(iii) *Dominance*

As is well known, most mutants are recessive to their wild-type allelomorphs. It is of interest, therefore, to determine the dominance relation between two allelomorphs, each derived from the wild type of a different species.

It is reasonably certain that the wild types of none of the parent species of *Verbena* contained any of the following allelomorphs: **a**, **ye**, **u**, **c**, **s**, **w**, **z**, **g**, **n**, **x**, since all have a striking effect on the plant and none of the wild species has an appearance resembling that of plants containing these allelomorphs. It is probable that they have arisen by mutation from allelomorphs contained in the wild species. The dates of some of these mutations can be surmised, e.g. **C**→**c** about 1870 (see above, § 2). Every one of these mutants is completely recessive.

Considering the remaining pairs of factors, i.e. those which can be traced back to differences between the original species, the dominance relations are less clear.

The allelomorph **d** has been shown to occur in the species *V. platensis*, while all other species are fully coloured and would therefore be expected to carry the allelomorph **D**. In the garden strains, the dominance of this factor is definitely intermediate (see Pl. XIII), though the heterozygote is in appearance more like **DD** than **dd**.

The character "long spike" was also derived from *V. platensis*. There is however, a considerable amount of variation in length of inflorescence among the other species, *V. peruviana* having the shortest and *V. incisa* and *V. phlogiflora* being intermediate. The inheritance of spike length is too complex for the dominance of factors controlling it to be decided, but at least one of the allelomorphs derived from *V. platensis* (**i**) is apparently recessive in the garden strains.

V. platensis contains the allelomorph **e**, as shown by test mating, though the flower colour in that species is too pale for the eye itself to be observed. *V. peruviana* has a very small eye, while *V. incisa* and *V. phlogiflora* have medium-sized eyes like the **E** type of the cultivated strains. While in some families there is practically complete dominance of "eye" over "eyeless", the size of the eye varies considerably. Whether

this is due to incomplete dominance of the factor **E** or to modification by other factors is not known.

The allelomorph **R**, derived from *V. platensis* or *V. peruviana*, is probably incompletely dominant to **r**, derived from *V. incisa* or *V. phlogiflora*, though the presence of modifiers makes it difficult to determine accurately all the genotypes.

The origin of the factors controlling the difference between purple and dominant or recessive scarlet is obscure. *V. peruviana* is the only scarlet species, but the available material of it consists of a clone, completely sterile; consequently one cannot test whether it contains the allelomorphs **P^dM^d**, **pm** or others. *V. incisa* and *V. phlogiflora*, as figured, have a scarlet-magenta colour, while *V. platensis* contains a pigment (delphinidin 3 : 5-dimonoside) the same as that in the cultivated purple forms.

The existence of two such series of multiple allelomorphs as **M^d-M-m** and **P^d-P-p**, in both of which the "extreme" members produce identical phenotypes, is a very unusual phenomenon, and may be related to the interspecific origin of the material. (It may be pointed out that homologous wild-type genes from different species are known to form multiple allelomorphic series, e.g. in *Antirrhinum*, **Ros_{uni}**, **ros_{dor}**, etc. (Kühl, 1937) and *Gossypium*, **L^A**, **L^N** etc. (Silow, 1939).)

It is also surprising that scarlet-magenta may be dominant to purple, because the difference between these two colours is due to an anthocyanin modification which occurs frequently in varieties of other plants, where it is almost always found that anthocyanins of the purple-*Verbena* type are dominant to those of the scarlet-magenta-*Verbena* type. This will be further discussed in the second paper of this series.

With regard to dominant and recessive maroons, there is less evidence from similar chemical changes in other plants, but from the existing data it would be expected that maroon would be recessive to purple.

Two other examples of multiple-allelomorphic series, similar to those occurring in *Verbena*, have been described. In maize, Emerson & Anderson (1932) report that, with certain combinations of other factors, there are both dominant and recessive allelomorphs for brown, as distinct from red, pericarp colour; i.e. **A^b** = brown, dominant to red, **A** = red, and **a** = brown, recessive to red; but **A^b** can apparently be distinguished from **a** by an effect on the colour of the plant and aleurone. In cotton, Harland (1939, p. 137) shows that there are both dominant and recessive factors, allelomorphic, inhibiting hairs on the seed. This example is very similar to that in *Verbena*, since the dominant naked factor **N²** in cotton was derived from *Gossypium hirsutum*, while the other two allelomorphs,

tufted T^B and recessive naked t^B were from the related species *G. barbadense*. Evidence will be brought forward (Beale, Price & Scott-Moncrieff, unpubl.) suggesting that in *Verbena* the allelomorphs M^d and P^d have been derived from *V. peruviana*, M and P from one of the other species and m and p by mutation from M and P respectively.

The results from *Verbena* on the dominance relations of interspecific and intraspecific gene differences agree well enough with the data so far obtained in other organisms. Thus, many recessive mutant genes have been transferred to nearly related species or genera, and remained recessive in the new genetic environment, e.g. in *Antirrhinum majus* (Kühl, 1937), *Drosophila melanogaster* and *Zea Mays* (data collected by Harland, 1936) and *Lycopersicum esculentum* (Afifi, 1936). Examples like the crinkled-dwarf in cotton (Harland, 1936), where dominance modification takes place on transference from one species to another, are probably exceptional.

It is commonly found that when two species are crossed, the hybrid is intermediate in respect of the distinguishing characters. There are two possible mechanisms for this: (1) incomplete dominance, and (2) two or more pairs of genes controlling the given difference, with at least one recessive and one dominant allelomorph in each species. Possibly both mechanisms act together). There is very little evidence on this question. Dahlgren (1924) found that in crosses between the reddish brown *Geum rivale* and the whitish *G. pallidum*, the F_1 was intermediate in colour and in the F_2 a 1 : 2 : 1 ratio was obtained for reddish brown : pale : whitish. Similarly with barley (see Matsuura, 1933) a number of investigators have obtained 1 : 2 : 1 ratios in F_2 's derived from crosses between two-rowed and six-rowed species, though there are other complications. Both these examples are, however, unsatisfactory because the differences between the parents are scarcely great enough to be considered as species differences.

It may be concluded that the evidence from both *Verbena* and other sources, slight as it is, indicates that incomplete dominance is liable to result when two allelomorphs are derived from the wild types of two different species, but that mutants are usually recessive and remain recessive when introduced into other species. Further work is required on this problem.

(iv) Linkage

Although no systematic attempt to determine linkages of all the factors has been made, a few striking examples of tight linkage have been

discovered. At one locus there are the three allelomorphs M^d , M , m linked to the factor c with $0.23 \pm 0.13\%$ crossing-over, and to the factor u with $<0.82\%$ crossing-over. In another chromosome there are the three allelomorphs P^d , P , p closely linked with the factor s , though the numbers are insufficient to allow of the calculation of an accurate recombination value.

These tight linkages are thought to be due to the presence of chromosomal irregularities, such as inversions, which are commonly found in interspecific hybrids and which reduce the frequency of observed crossing-over in adjacent regions. The only stage of meiosis observed in *Verbena* was metaphase, but there univalents occurred frequently. Anaphases have not been seen, and therefore the presence of chromosome bridges formed as a result of crossing-over between inverted segments could not be recorded.

6. SUMMARY

1. The garden forms of "*Verbena hybrida*" show variations of two kinds, the first apparently derived from differences between its four reputed parental species, the second from mutations which have taken place since the original hybridization about a hundred years ago.

2. The second group depends on ten gene differences, which show complete dominance of a common wild type. The first group on the other hand depends on eight gene differences which show various degrees of dominance.

3. There are two sets of triple allelomorphs controlling colour, which are remarkable in that the extreme members of each series determine the same phenotype and differ only in that one is dominant, the other recessive, to the intermediate allelomorph. Possibly the dominant member is derived from a species difference, the recessive from mutation.

4. Aberrant ratios, modifying factors and extremely close linkages occur with exceptional frequency and are probably due to the hybrid origin of the group.

The pigment chemistry will be described later.

I would be most grateful if collectors or others in South America would supply seeds from the wild of any species mentioned in this paper, or any other closely related species.

REFERENCES

- AFIFI, A. (1936). "Some evolutionary aspects of a comparative cytogenetic investigation between *Aconitum* and *Solanum*." *Genetica*, **18**, 255-76.
- BAILEY, L. R. (1902). *Cyclopedia of American Horticulture*, **4**.
- DAHLGREN, K. V. O. (1924). "Kreuzungskleinigkeiten. Versuche mit *Geum rivale*." *Hereditas, Lund.*, **5**, 222-30.
- DE CANDOLLE, A. (1847). *Prodromus systematic naturalis*, **11**, 535-57.
- DERMEN, H. (1936). "Cytological study and hybridization in two sections of *Verbena*." *Cytologia, Tokyo*, **7**, 160-75.
- EMERSON, R. A. & ANDERSON, E. G. (1932). "The A series of allelomorphs in relation to pigmentation in maize." *Genetics*, **17**, 503-9.
- EMSWELLER, S. L. & BLODGETT, C. O. (1937). "Inheritance studies of a dwarf mutant in *Verbena*." *Proc. Amer. Soc. hort. Sci.* **35**, 822-4.
- HARLAND, S. C. (1936). "The genetical conception of species." *Biol. Rev.* **11**, 83-112.
- (1939). *The Genetics of Cotton*. London: Cape.
- KÜHL, O. (1937). "Genanalyse bei *Antirrhinum*-Artbastarden." *Z. Indukt. Abstamm.-u. VererbLehre*, **74**, 125-60.
- LECOCQ, H. (1852). "Culture des Verveines comme plantes annuelles. *Rev. hort.* **4**, 5-12.
- MATHER, K. (1938). *The Measurement of Linkage in Heredity*. London: Methuen.
- MATSUURA, H. (1933). *A Bibliographical Monograph of Plant Genetics*, 2nd ed. Sapporo.
- NOACK, K. L. (1937). "Die Chromosomenzahlen einiger *Verbena*-Arten." *Biol. Zbl.* **57**, 383-88.
- SILOW, R. A. (1939). "The genetics of leaf shape in diploid cottons." *J. Genet.* **38**, 229-76.
- WINGE, Ö. (1937). *Arvelighedslaere*. Copenhagen.

EXPLANATION OF PLATES XIII—XV

PLATE XIII

Flowers

- | | |
|---------------------|-------------|
| 1. Purple. | 4. Scarlet. |
| 2. Maroon. | 5. Plum. |
| 3. Scarlet-magenta. | |

All purple

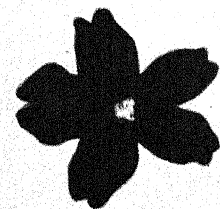
- | | | |
|--------|--------|--------|
| 6. DD. | 7. Dd. | 8. dd. |
|--------|--------|--------|

PLATE XIV

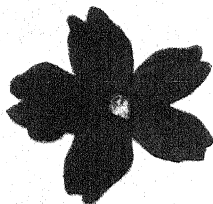
- | | |
|-----------------------|----------------------------|
| 9. C and c plants. | 11. g—"less extreme" type. |
| 10. g—"extreme" type. | 12. G—normal. |

PLATE XV

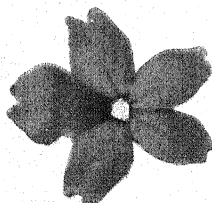
- | | |
|-------------------------------|-------------------------------|
| 13. Medium eye E. | 17. "Normal" inflorescence I. |
| 14. Coloured rim round eye R. | 18. Long spike i. |
| 15. Eyeless e. | 19. Normal petals W. |
| 16. Very large eye. | 20. Wrinkled petals w. |



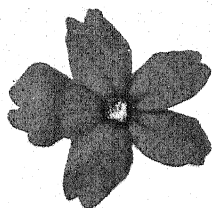
1



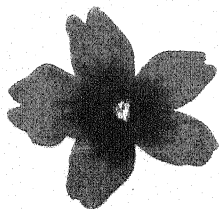
2



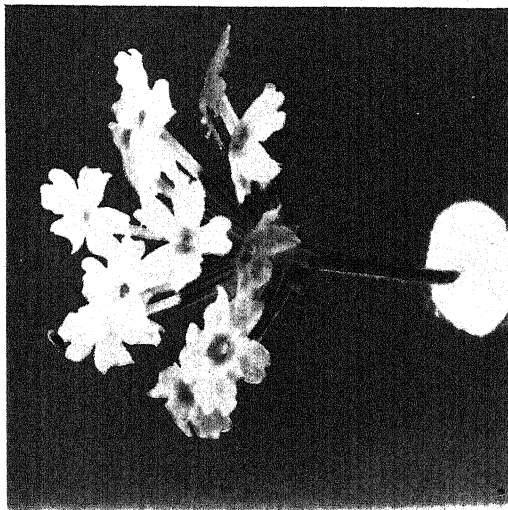
3



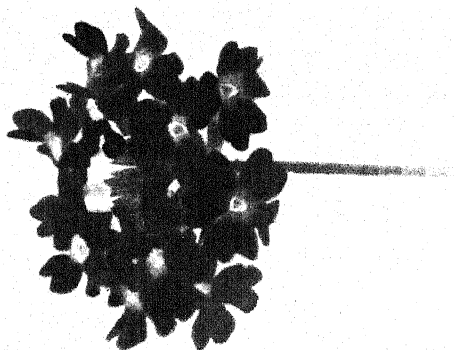
4



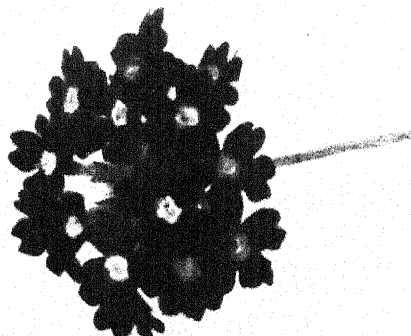
5



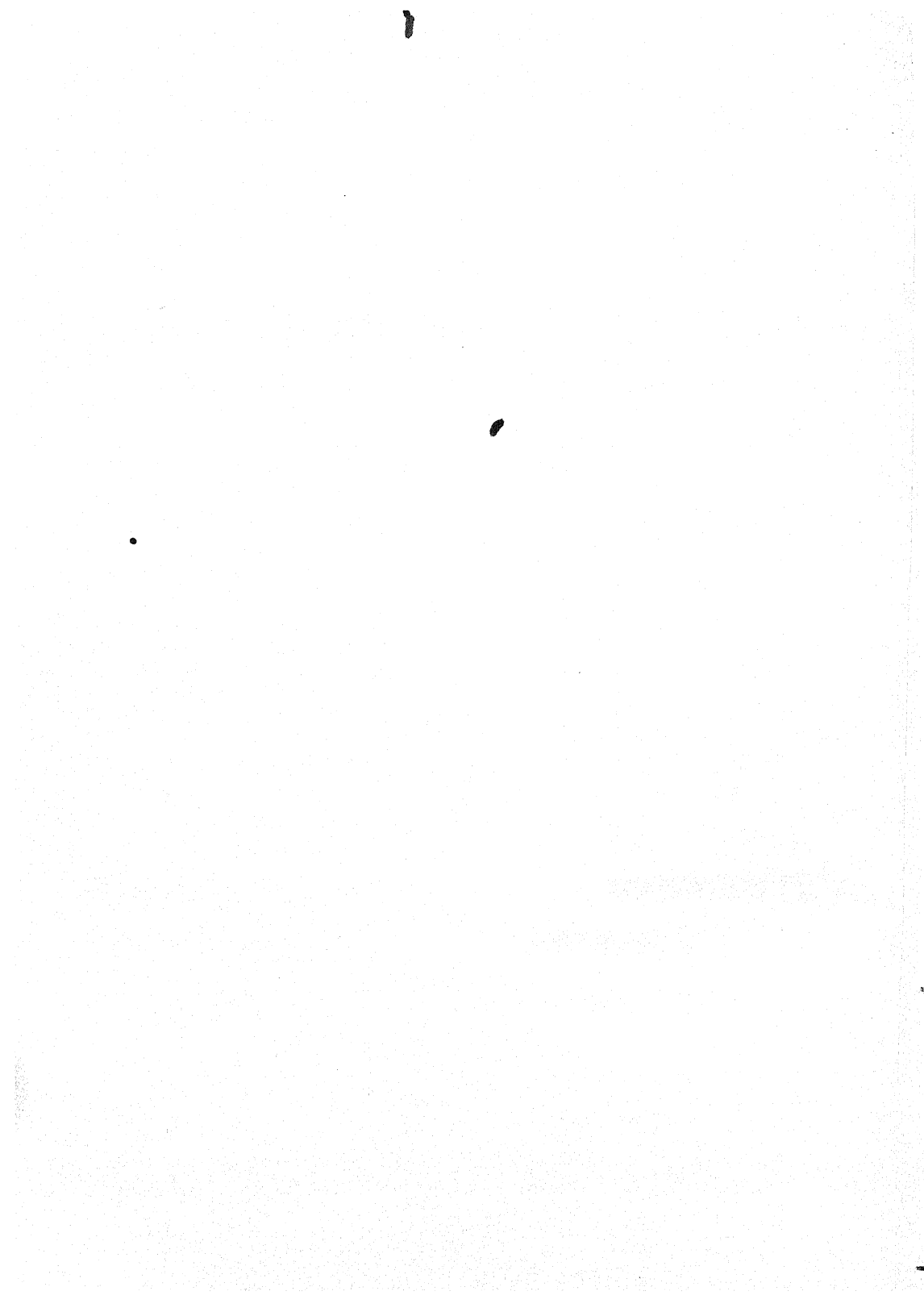
8

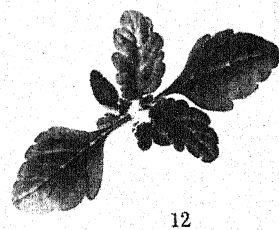
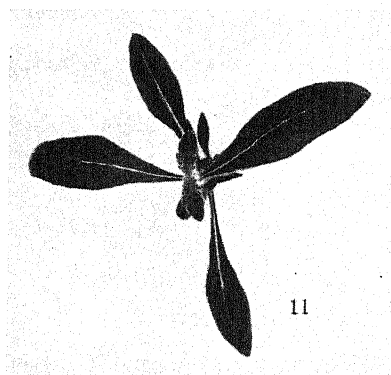
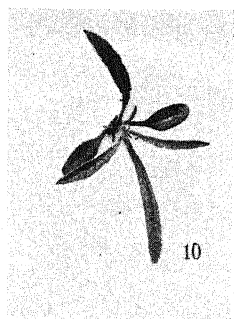


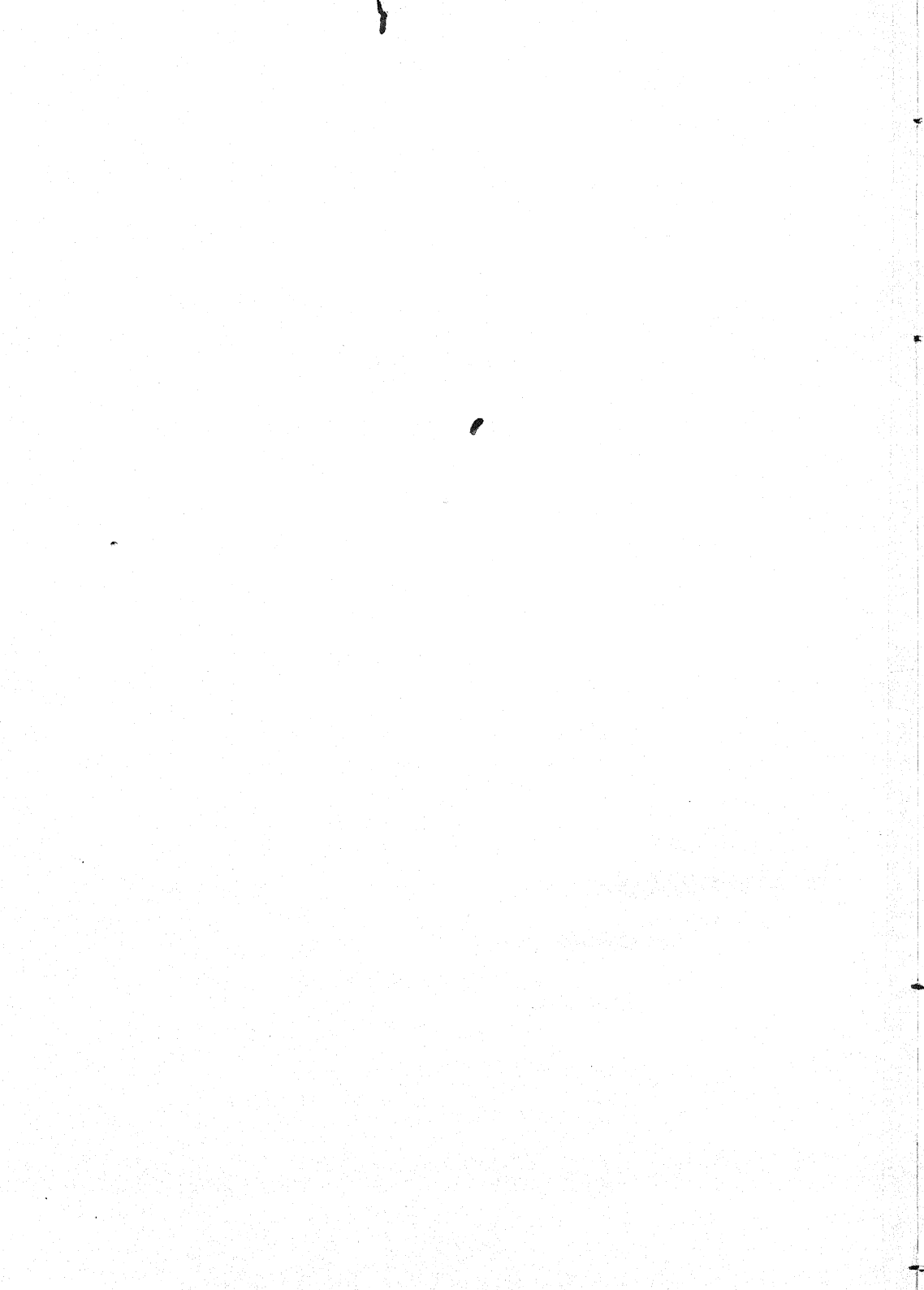
7

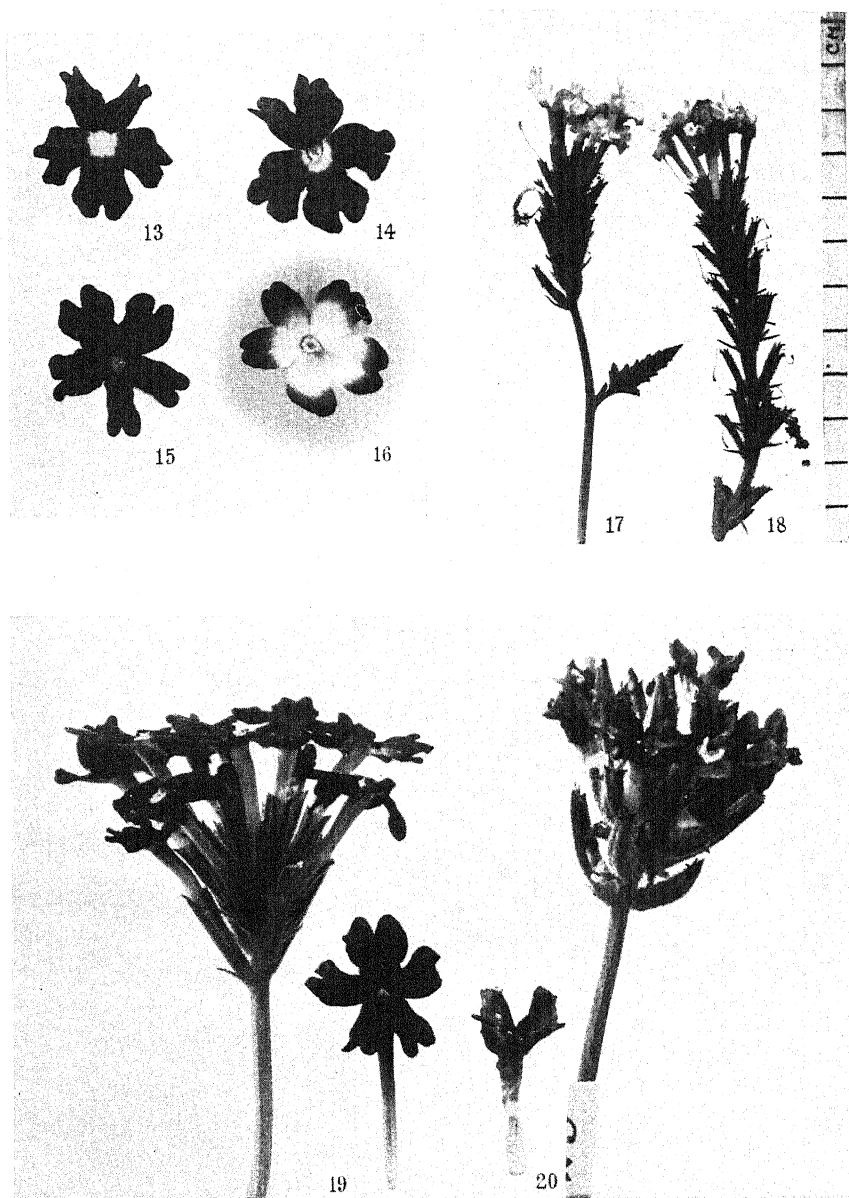


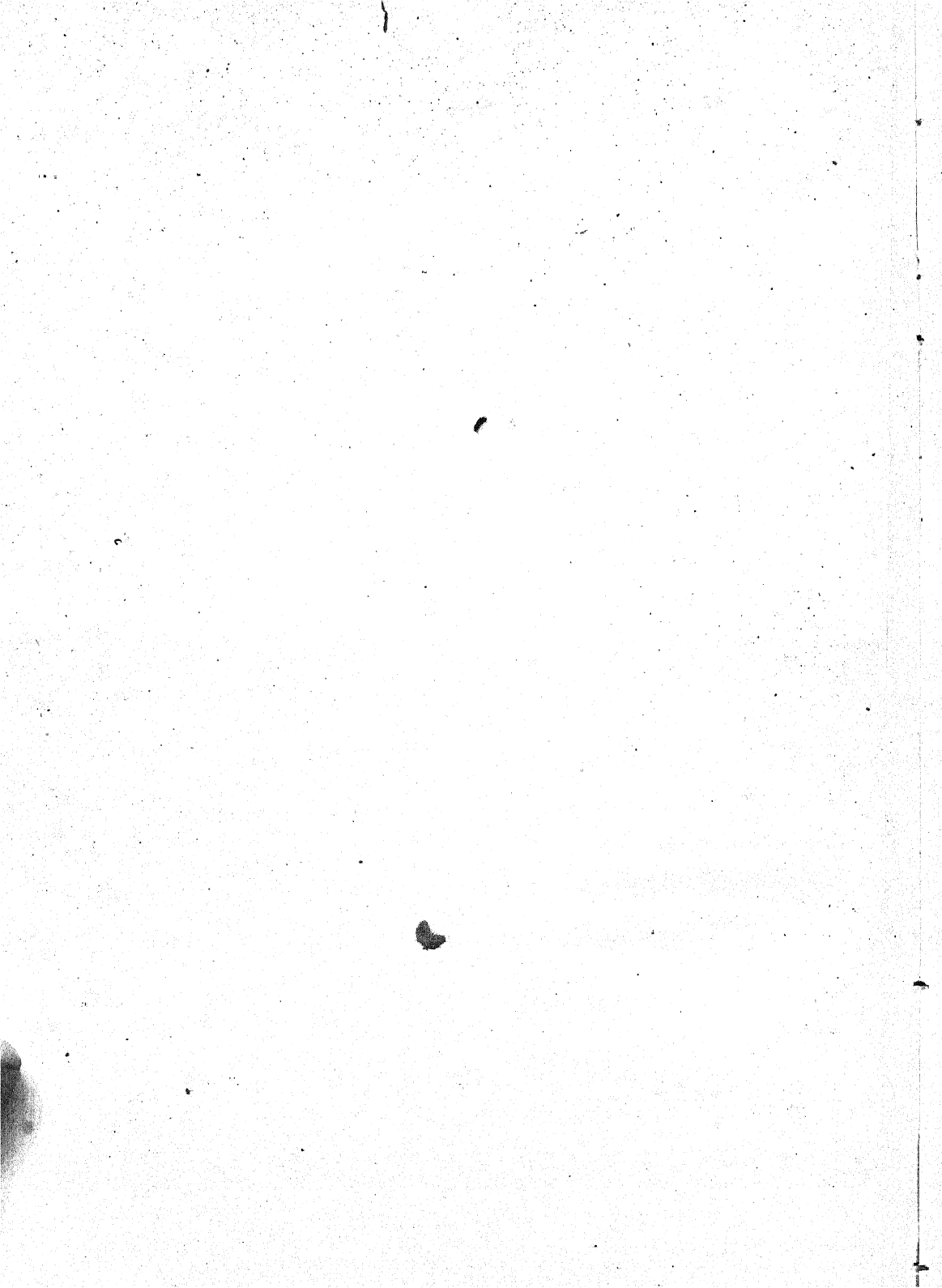
6











THE FABRIC STRUCTURE OF PROTEINS WITH SPECIAL REFERENCE TO CYTOGENETICS

By DOROTHY WRINCH¹

THERE can no longer be any doubt that certain proteins, such as pepsin, insulin, the haemoglobins, etc., like other substances which are chemically less formidable, have definite structures (Svedberg, 1939). An early hint (perhaps the first) of this specifically modern point of view is contained in the work on proteins published by Schimper (1881). The final proof is to be found in the present vast stores of data belonging to enzyme, hormone and virus chemistry and immunochemistry (Heidelberger, 1938). Particularly striking are the facts belonging to the general fields of physical chemistry (Cohn, 1938), where studies have been made of the physical properties of the most varied proteins in terms of osmotic pressure, viscosity, dielectric constant, electrophoretic migration, differential solubility in water and alcohol, their combination with acids, bases and metallic ions, their molecular weights and the new and important property of dissociation. Of paramount importance is the fact that, in certain circumstances, some proteins are isodisperse in solution.

Indeed, there seems to-day to be a clear case for the view that vast series of proteins are built of particles having "the hall-mark of individuality", to use Svedberg's phrase. It now seems justifiable and even necessary to picture a great variety of proteins as organic substances having structures as definite as any known to chemistry: the crystalline-soluble proteins in fact are now regarded as having molecular status in a certain definite sense, and they constitute indeed the first megamolecular structures. These structures, Svedberg suggests, are built according to a plan which makes every atom indispensable in the complete structure. In particular, the property of dissociation of proteins in solution into smaller units has been studied intensively. It is found that this phenomenon can be induced by dilution, by a change in *pH*, by the introduction of foreign molecules, such as urea, clupein, arginine, etc., and it has been concluded that many, even possibly all of the heavier proteins are formed by the linking together of smaller units, also having the protein character. The interlinking may be due to bonding together of *R* groups, bonding of

¹ Prepared at the invitation of the Organization Committee of the Seventh International Congress of Genetics, for the session on protein and virus studies in relation to the problem of the gene.

these or other atoms in their structures by means of hydrogen or hydroxyl bonds, or even to their being held together by foreign constituents. It is convenient to talk of protein units when we mean the structures having protein character into which such proteins can be dissociated by these various techniques, and to talk of protein colonies when we mean the composite structures, capable of reversible dissociation. Much attention has of course been directed to the molecular weights of protein colonies and protein units. These cover an enormous range. They range from the very large molecular weights which have been derived for the haemocyanins and virus proteins, to weights as small as nine or ten thousand. The molecular weights of even the smallest protein units thus appear to be large in comparison with the weights of the molecules of present-day chemistry. Indeed the fundamental question for protein chemistry (Wrinch, 1937 *b*, *c*, 1938 *d*) is to explain the existence of megamolecules, i.e. very large but chemically and physically well-defined units containing hundreds or even thousands of amino (and imino) acid residues. These, it appears, are compact and are built on some general plan which accounts for their common properties, the various individual selections of the various residues being arranged in definite interarrangements which give to each its own highly specific individuality and personality.

It should be realized that this problem of the megamolecule is a new problem for physics and chemistry. The last decades have seen an enormous interest in long-chain molecules, the so-called macromolecules. By means of different techniques it is now possible to synthesize such chain polymers with a variety of different compositions. Though they are of course of definite chemical composition, they are not chemical individuals. Rather they are collections of polymers of roughly the same length. They are macromolecular in the sense that they have enormous weights, but the particles to which these enormous weights refer are not definite and individual structures.

Comparison of the physical properties of these macromolecules and the protein megamolecules at once discovers the fundamental differences between them. A simple illustration will suffice. A study of the dielectric properties of the poly- ω -hydrodecanoic acids shows that the contribution of these polymers per gram to the dielectric constant is the same for all different chain lengths, and no fall in dielectric constant with increasing frequency is observed (Oncley, 1938). Apparently these polymers are loose chains in which each section can rotate independently. With certain proteins, on the other hand, it is found that the whole structure rotates as a unit. There is apparently a fundamental difference

between the two types of structures: the one is a straight chain without rigidity, whereas the other is bound up in some way so as to rotate as a whole. Many other differences have come to light in recent years. They can all be summed up by insisting on the fact that these proteins have in some sense highly definite individualities and personalities, which are shown by their power to crystallize, their specificities in biological reactions, and other properties, all of which may be connoted by the term megamolecule.

The current belief when I began my studies of chromosome structure in 1934 was that the essential entity in proteins is a linear sequence of residues. As a newcomer to the field, I adopted this view as the basis of my picture of chromosome structure (Wrinch, 1936 *a*). My subsequent studies in protein chemistry and physiology and crystallography, however, soon made it clear to me that such a type of structure does not account for the established facts (Wrinch, 1936 *b, c*, 1937 *a, b, c*, 1938 *a, b*, 1939 *a*). The special and definite molecular weights of the globular proteins are difficult to reconcile with a chain structure, especially in view of the rather clear and detailed knowledge now existing as to the nature and behaviour of the long chain molecules in general (Langmuir, 1939 *b*). It is hardly an exaggeration to say that no two classes of substances seem to differ more widely in all their properties. Another outstanding characteristic of these proteins, namely, their capacity to form, spontaneously from solution, highly insoluble monolayers, is also in conflict with such a view. Most important of all, the capacity of such proteins to denature betokens a structure very different from that of the long-chain molecules which do not exhibit even a hint of such behaviour. For these and other reasons (Langmuir, 1939 *b*) a new analysis of the data relating to proteins seemed an urgent necessity, with the special object of seeing how far the accepted facts require or imply or even suggest in themselves this picture of proteins as linear molecules which had dominated the field of protein chemistry for so long.

That the proteins are substances of definite chemical *composition* has not been in question since the time of Fischer and Hofmeister, whose researches, while leaving open the question of structure, permit the deduction that the protein substances are polycondensation products of numerous varieties of this specific species of building block, namely, the amino-acid and the imino-acid molecules. In point of fact Fischer and Hofmeister summed up their researches by postulating that proteins are polypeptide chains which constitute one special type of polycondensation product, namely, open linear polycondensations. But the chemical

analyses upon which their conclusions were based related only to the composition of proteins and not to the structure: information regarding this comes more naturally from crystallographic investigation on the one hand, and researches in enzyme, immuno- and physical chemistry on the other. For many reasons, plain from a study of the data in these fields of protein work, it is preferable (as it is certainly permissible) to restate their conclusions in the wider form that proteins are polycondensation products of amino-acid and imino-acid molecules. It is then necessary to formulate the problem of protein structure as the study of all permissible polycondensations of the given building blocks, an investigation essentially of a geometric nature (Wrinch, 1938 *a*).

In calling geometry to the service of this branch of megachemistry, a new point of view is necessarily introduced which has as its objective the formulation of all possible types of structure. The gradual development of this point of view has brought many results, surprising or perhaps only novel, notably the realization that geometrical considerations alone can go far towards determining characteristics which very complicated chemical structures must have.

The original idea of the amino-acid residue as a two-armed unit $-(\text{HN}-\text{C}_\alpha\text{HR}-\text{CO})-$ was used by Fischer and Hofmeister as the basis of their theory, and in consequence they postulated that the polypeptide chain is the essential entity in protein structure. However, this two-armed unit can also be built into a cyclopolypeptide, and it has accordingly been suggested that the polypeptide ring (i.e. the anhydride of a polypeptide chain) may be an element in protein structure (Wrinch, 1936 *b, c*, 1937 *a*, 1939 *a*). If we rewrite the residue in the form $\text{>N}-\text{C}_\alpha\text{HR}-\text{C}(\text{OH})\text{<}$ we have in addition a four-armed building unit which, being only a prototropic modification of the two-armed unit, may at all times be in tautomeric equilibrium with it. The polypeptide ring, capable of intra- and intermolecular prototropic tautomerisms and hydrogen bonding (and possibly in addition capable of interlinking by means of *R* groups), seems to be capable of explaining all the phenomena of protein monolayers so far discovered, including their elasticity, viscosity, capacity to form fibres, multilayer structures and so on (Langmuir, 1938 *b*, 1939 *a*). In accordance with a simple geometrical scheme, such polypeptide rings are geometrically capable of forming two-dimensional networks of atoms (the cyclol fabrics) with which they can be in tautomeric equilibrium (Wrinch, 1936 *b, c*, 1937 *a, b, c*, 1938 *a, d*, 1939 *a*). Other fabrics involving hydrogen bonding have also been con-

structured (Wrinch & Jordan Lloyd, 1936). It has therefore been suggested that the essential entity in the biologically active proteins is not a linear or chain structure but a fabric which carries the *R* groups in a definite pattern. We may regard the ground-plan of this fabric as the common characteristic of all proteins: and we may regard the innumerable particular patterns in which individual selections of *R* groups can be arranged on the fabric as the special characteristics of individual proteins. Such a picture suggests that not all selections of different *R* groups are equally favourable, since definite restrictions on individual patterns are to be expected. In general, it may be anticipated that the numbers of individual varieties will have reference to the types of symmetry of the protein ground-plan. This principle of preferential proportions of certain amino-acid varieties in proteins explains many facts relating to the chemical composition of proteins as different as egg albumin and keratin, haemoglobin and fibrin: moreover, the fact that a number of varieties occur persistently in proportions involving powers of 2 and 3 evidently indicates symmetries of these types in the ground-plan of proteins in general. This fact is evidently capable of interpretation in terms of the cyclol fabrics, since these fabrics have locally symmetries 2 and 3. But the important point to stress is not these particular fabrics but the more general and fundamental idea of an atomic fabric or skin as possibly the important and essential entity in protein structures (Wrinch, 1938 *d*). The cyclol fabric is a direct outcome of realizing that the amino-acid and imino-acid residues need not be regarded simply as a two-armed unit capable, in consequence, of forming only linear structures, i.e. open chains or closed chains or rings. They can also be regarded as units with a larger number of arms (Fig. 1). Evidently the possibility of hydrogen

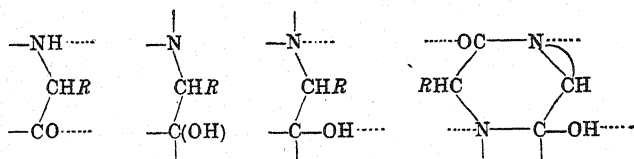


Fig. 1.

bonding gives the building block four or more arms, and we would emphasize that fabrics of this type have already been devised. Provided any building block has more than two arms, it can be built into a structure which is two- or even three-dimensional.

Now the possibility of specific bondings of residues by means of special *R* groups (e.g. arginine with glutamic acid and cystine SS bridges)

has of course been in the minds of protein chemists for a long time. The work of Astbury (1938), who introduced this idea in his keratin structures, is very well known. That it may play some part also in the structure of the globular proteins must be borne in mind. But the essential protein character is shared by substances having such very different complements of *R* groups capable of such interbondings that it seems difficult, indeed impossible, to attribute it wholly or even mainly to such linkages. Rather, I suggest, we should follow the intuition of Fischer and Hofmeister who felt compelled to look for the essential protein character in bondings of atoms which are common to all the building blocks, namely the skeletal atoms, H, O, C, N. The idea of peptide links concentrates attention on interbonding of residues by joining the N of one to the terminal C of another. This suggestion, which in the original polypeptide chain hypothesis is confined to bonding each residue at each terminal to one other residue at most, can be extended, as in the cyclol hypothesis, to possible bonding of each residue at each terminal to one or other of two residues, still by means of N—C links. It will be noticed that four such bondings of each residue, two at each terminal, by means of the N—C bonds suggested by Fischer and Hofmeister, can be accomplished simply by means of a prototropic transfer either within one residue or from one residue to another. In the special case of the imino acid residue a similar prototropic transfer between residues allows correspondingly a three-armed unit. The special attributes of cyclol fabrics containing imino residues are being studied.

We have already pointed out that the essence of the geometrical approach to protein structure is a consideration at every point of ALL possible structures. There is of course necessarily a great difference between this approach and that forced upon those workers who study special techniques. Those who work in the physical chemistry of proteins, e.g. in electrochemistry, have as their objective the discovery of and the exact measurement of the physical parameters appropriate for the description of the material under study, and the great art in such researches is to discover the physical parameters which throw the maximum amount of light on structural problems. In the first instance, however, all physical parameters are the objective. Similarly, in protein crystallography, the first objective is the collection of experimental facts relating to the sizes and shapes of unit cells and to the lengths and orientations of specially important distances in the crystals. In the early stages of protein chemistry or any other discipline, it may be very difficult to find direct bridges between the two points of view and much valueless contro-

versy is likely to arise when there is an inadequate appreciation of the difference in the immediate objectives of the two types of investigation (Wrinch, 1936 *b*, *c*, 1937 *a*, 1939 *a*).

The situation regarding the nature of the polypeptide fabric, which I suggest may be the essential entity in protein structure, provides an instructive example of the geometrical point of view. The cyclol link, it was found, in itself provides a complete system of structures which have characteristics which accord remarkably well with many of the qualitative aspects of protein chemistry. But it is of equal importance to put forward for consideration any and every type of polypeptide fabric consistent with the chemical facts. It is for this reason that, without prejudice to the cyclol hypothesis, it seemed to me useful also to work in detail on the possibility of formulating the building blocks of proteins in the forms shown in Fig. 1. These, which follow the suggestion of Dr Jordan Lloyd in 1932 (Jordan Lloyd, 1932, 1933) also allow the construction of polypeptide fabrics since they give to the individual residues more than two arms. Some of the possibilities suggested by both this idea and the cyclol idea are shown in Fig. 1. Any one of these alone or in combination with others can be studied (Wrinch, 1939 *b*). The resulting fabrics, some of which were worked out by Dr Jordan Lloyd and myself in 1936, are at present under consideration.

Many of the special features to which the cyclol fabric has directed attention are also potentially present in these other fabrics. I would mention particularly the special symmetries which are present and the way in which they offer an interpretation of the possible significance of the two outstanding uniformities of the residues in proteins, namely, the fact that the building blocks have a C—C—N skeleton and not a C—C—C—N or C—C—C—C—N skeleton, etc., and the fact that the configuration of the central C atom is always the same, giving residues of the so-called laevo type.

My first point is therefore that the proved and accepted composition of the simple proteins as polycondensation products of the amino and imino acids leads to the consideration not only of linear peptides which are open but also of linear peptides which are closed (i.e. peptide rings) and also of peptide fabrics in which the residues are built into a crust or skin (Wrinch, 1938 *a*). Such a skin or crust of residues has of course the correct composition and, provided it can fragment into linear peptides, satisfies all the chemical facts.

Once we realize that a fabric structure for proteins is consistent with the known facts obtained by chemical analysis it becomes of great

interest to see how far this idea allows us to build a picture which fits such important additional data as the capacity of proteins to form insoluble monolayers, their capacity to denature in general, and, most important of all, the outstandingly important conclusion that such proteins are chemical individuals having very definite personalities, and incidentally definite molecular weights (Langmuir, 1939 *b*).

The study of the open fabric at once suggested the possibility of making closed structures by folding (Wrinch, 1937 *b, c*). Instead of trying vainly to make a chemical personality out of long-chain polymers which apparently always exist in a number of chain lengths grouped about some average length, we have presented to us the quite different picture of a closed structure or atomic envelope—for example, a polyhedral cage or a polyhedral anchor ring (torus)—in which the geometry itself goes far towards determining the possible numbers of building blocks in each skeleton. We then come upon the idea of residue number as a basis of classification of the globular proteins (Wrinch, 1936 *b, c*, 1937 *a*, 1939 *a*), an idea which explains how proteins with the most varied selections of residues can yet have the same molecular weight. The lack of complete chemical analyses of proteins necessarily makes it impossible to deduce with any certainty the number of residues in this or that protein, though an attempt in this direction has been made by Bergmann and Niemann. But, if the situation is that suggested by these geometrical considerations, it is to be expected that the molecular weights of proteins will not spread more or less uniformly over the whole range of weights, but will tend to fall into favoured ranges, and that, far from the molecular weight being in any sense characteristic of a preponderance of this or that type of residue, proteins with somewhat similar molecular weights may be found which in their individual selections of the various types of residue differ widely.

This very simple idea for the first time offers a possible explanation of the central fact of protein chemistry, namely, that a structural unit which has its own definite personality as shown by its physical and physico-chemical behaviour can be formed, not only from a few atoms as in the familiar molecules of present-day chemistry, but also from hundreds or thousands of atoms. As an example we may cite the cyclol structure which in its skeleton is made up of seventy-two residues (all amino acids). Evidently the deletion of even one of these residues will disturb the whole structure.

This idea of a characteristic protein fabric is of course capable of development in a large number of different ways, as is shown by the several

forms in which the building blocks themselves may function (Wrinch, 1938 *d*). But common to all these is the fundamental characteristic of explaining the existence of megamolecules as due to a closed atomic envelope. It is plain that simple arithmetical relations may be expected between the various numbers of residues which form the skeletons of such series of structures. We see, for example, that the size and shape is determined by the fabric, and any one size and shape will necessarily suggest for consideration a complete homologous series, determined by the pattern in the fabric itself. In this way one could interpret series of globular protein whose molecular weights indicate that their residue numbers are multiples of certain basic numbers (Wrinch, 1938 *b, c*). This situation, it is claimed by Svedberg, has already been realized.

On the other hand, the dissociation of so many of the heavier proteins into smaller protein units may mean that all proteins are in fact made up of a very few, perhaps even only one or two, protein structural units. In such circumstances, the association of such units into colonies will itself explain the arithmetical relations which Svedberg postulates between protein molecular weights. Intriguing as these questions are, I suggest that the outstanding achievement of these physicochemical techniques is the proof that, whether as units or as colonies of units, a vast number of the globular proteins are chemical individuals. And I suggest that this individuality of these structures is in essence understandable in terms of the idea of closed fabric structures.

An important point about a fabric structure as opposed to a linear structure is the multiple paths of connexion between any two atoms (Wrinch & Langmuir, 1939). In a chain structure, different substituents on atoms influence each other mainly through the single line of intermediate atoms. With a fabric, every two atoms are multiply connected and even rather remote groups may therefore have an influence on one another which is far greater than their distance apart would warrant if only one intermediate series of atoms existed. Evidently in the case of a closed fabric this situation is accentuated, for those atoms which are farthest removed from one another may be brought into intimate contact when the fabric is folded round to form a closed envelope. This characteristic of fabrics has been stressed as of special significance for proteins, since in these structures, above all, there seems to be an intimate interaction between various constituent residues and atoms. The possibility of resonance in such envelopes has already been suggested, and this may turn out to be the most important argument in favour of closed envelopes and against chain structures (Wrinch, 1939 *b*).

All these considerations apply to all atomic fabrics which have been put forward for consideration (Wrinch, 1938 *d*). It is necessary only to make sure that the composition of the fabric is of the known protein type and that it is capable of fragmentation into linear peptides.

To make the situation clearer we may cite the situation with the cyclol fabric, with which it is possible to construct polyhedral cage structures containing 72, 288, ... residues in the skeletons (Wrinch, 1936 *b, c*, 1937 *a, b, c*, 1939 *a*). These have been constructed under very strict conditions, namely, that all the C and N atoms are strictly tetrahedral in their valency angles and in addition only amino-acid residues have been allowed. As opportunity offers, these metrical conditions (which it appears may be unnecessarily stringent) are being relaxed and new structures including also imino-acid residues have been devised. Evidently the polyhedral character of these constructs and the fact that such structures may be electronically unsaturated suggest that cage colonies containing two or more of these cages in association may also exist, thus explaining the property of reversible dissociation of protein molecules which has been discovered in the ultracentrifuge. In this way residue numbers which are multiples of 72 are predicted. These prove to tally with all the molecular weight classes found by Svedberg. In particular the number 288 was proposed as the residue number in the skeletons of proteins with molecular weights around 36,000 (Wrinch, 1938 *b, c*). Subsequently, it has been stated that chemical analysis of one of these, namely, egg albumin, permits the deduction that its molecule does contain the predicted number, namely, 288 residues.

The polyhedral character of these cage structures also explains in general terms the mechanism of megamolecular crystallization, since the apposition of plane faces, of edges or of slits allows various types of packing and yet shows how interbonding adequate to ensure stability can be facilitated. Further, the idea that the polyhedral skeleton has certain high symmetries brings into one single scheme the heterogeneous facts about crystal classes relating to insulin, pepsin, lactoglobulin, haemoglobin and chymotrypsin (Wrinch, 1938 *d*).

The suggestion of the atomic envelope also throws light on the capacity of such substances, even when highly soluble, to form spontaneously monolayers of prodigious insolubility. For an envelope allows a molecule to hide in its interior hydrophobic groups which, once the envelope is opened, can account for a very high insolubility (Langmuir, 1938 *a, b*). Still more important is the fact that a closed structure throws light on the phenomena of denaturation in general (Wrinch, 1938 *a*). For clearly

the puncturing or ripping open of an envelope of the types considered connotes in some sense a qualitative difference, and this may develop without necessarily requiring any change in chemical composition. Opening of a cyclol bond is simply a migration of a hydrogen atom, and no new atoms are taken up or given off. The breaking of a hydrogen or hydroxyl bond similarly involves no change in chemical composition. Furthermore, this collapse of the envelope can presumably take place in very many different ways. There may be a slight local rent, capable of being easily repaired. There may be a tearing of the fabric into a single open fabric. There may be a ripping open into various fabric fragments or even a complete collapse into linear strands. On this picture, denaturation may not involve any difference in chemical composition, nor even a change in molecular weight, nor need it be irreversible. Nevertheless a qualitative change in structure may be involved resulting in most drastic changes in physical and physicochemical parameters. The Chinese chemist Wu many years ago drew attention to what in his opinion is the outstanding point about the biologically active proteins, namely that they possess some peculiarity of structure which allows denaturation to take place. I submit that the atomic envelope provides a possible solution of this peculiarity and with it of the nature of denaturation.

The idea of a closed surface structure for a molecule containing hundreds or thousands of atoms thus gives a useful working model which is capable of explaining the megamolecular status of the globular proteins. Evidently it also brings in its train a number of problems which are of the utmost difficulty and upon whose solution the possibility of testing such hypothetical structures depends. I would specially refer to the difficulties of predicting the laws governing distances between multiply connected atoms which are not directly bonded. Abortive attempts have, for example, been made to disprove the cyclol hypothesis by considering the energetics of the distribution and the abnormally small distances between certain non-bonded atoms in the fabric. Such attempts, useless as they may be in their avowed purpose, are at least useful if they draw attention, once again (Wrinch, 1936 *b, c*, 1937 *a, b, c*, 1939 *a*) to an important problem for theoretical physics in the future, namely the nature and behaviour and energetics of atomic envelopes in general.

Now the first question for cytogenetics is to consider how far either of the current pictures of the structure of proteins is applicable to the gene. One is the picture of the biologically active protein unit is an atomic envelope, applicable, it seems to me, to such proteins as insulin, the serum proteins, the virus proteins: the other, the classical polypeptide

chain picture, which may be applicable to the proteins at the other end of the scale, namely, the fibrous proteins such as the keratins, which have apparently lost their specificity and their highly definite megamolecular character, and in so doing have left the metabolic cycle. In my original studies of gene structure (Wrinch, 1934, 1935 *a, b*, 1936 *a*), as a newcomer to the protein field, I necessarily took over the picture of proteins current at that time, namely, that of a polypeptide chain. Subsequent studies of the various types of biologically active proteins, however, seemed to me to point to a radically different type of structure which should explain their unique properties. These studies led to the formulation of the fabric structure for the biologically active proteins. A consideration of the gene as the repository of individual pattern for the organism, as the essential structural unit which largely controls development, seems to me to weigh the scales definitely in favour of postulating for the genic units in an organism a structure which is capable of the highest possible degree of specificity and individuality. Comparing and contrasting the inert and the biologically active protein molecules, there seems to me little question but that a structure more akin to the latter than to the former will prove appropriate for the genic units. The striking properties of the enzymes and virus proteins are if anything enhanced in the genic proteins. I suggest that in fact all the arguments which tend to show that a chain structure is not satisfactory for these protein types (Langmuir, 1939 *b*) tend also to discount the view that the genic units may have a chain structure such as has been suggested for the keratins. The final knock-out blow for the chain hypothesis is provided by the evidence of the individual personalities of the proteins and its failure to find any interpretation of denaturation or any reason for definite megamolecular weights or for definite complements and definite arrangements in space of the individual selections of residues. It then seems difficult to refrain from seeing in these arguments applied to the genic units an even greater cogency. It is surely necessary to postulate for these immensely important constituents of the organism a structure as definite as that of enzymes and virus proteins. I therefore suggest that the genic units, as far as the protein constituents are concerned, may consist of a fabric folded round to form some closed structure (Wrinch, 1937 *d*). This structure need not be a simple polyhedral cage. Any closed fabric structure, e.g. a polyhedral anchor ring, alone or in colonies, may be sufficient to give the properties required, so far as we can see at present in the light of the meagre data available for our guidance.

It should be emphasized that actual measurements of the genic units

give no way of deciding in favour of the compact or the fibrous structure. A measurement for the gene of the order of a few hundred angstroms is consistent with either globular or fibrous units. The chromosomes may perhaps be fibrous in the sense that the genes are arranged in linear array, and only in this sense. On this point particularly, definite experimental data at present lacking are eagerly awaited. Similarly with the actual chemical composition of the gene, in so far as it is known. It is generally assumed that the protein constituents in chromosomes in certain species are very strongly basic, though it must be remembered that the sperm contains other protein-like materials made up of tryptophane, tyrosine, cystine and other amino acids. Kossel, it will be remembered, classified the protamines which he extracted from a wide variety of fishes as mono-, di- and triprotamines containing some mono-amino acids, but in each case a preponderance of arginine, of arginine and histidine and of arginine, histidine and lysine respectively (Kossel, 1928; Block, 1938). Particular attention has been directed to clupein, a protamine from herring sperm, in which it is calculated, there are about 5 arginine residues to 2 monoamino acid residues (Linderstrom Lang, 1935). Assuming then that the protein in such chromosomes is that of this highly basic type, how does this enable us to decide between the globular and fibrous structures? It has also long been assumed—and the recent work of Casperrson and Schultz has offered confirmation of this—that the protein in chromosomes is associated with nucleic acid (Casperrson, 1936). If this is so, how do these suppositions fit with the picture we now suggest of the genic units as atomic envelopes?

Now in the first place a closed protein fabric structure carrying a sufficiently high proportion of basic *R* groups may in itself be intrinsically unstable owing to an excessive charge per square angstrom of surface. But such an envelope can presumably be stabilized by the presence of an adequate concentration of charged groups of opposite sign. These, in this case, are presumably provided by the nucleic acid molecules known to be present. Just as in my first suggestion as to the structure of chromosomes, I view the nucleic acid as stabilizing the highly basic protein component. Just as in that hypothesis, I suggest that a balance between the two depends on a special value of the *pH* of the medium and can be upset by even a slight shift. A shift to one side requires an increase of one partner to balance the original complement of the other partner, and the return shift correspondingly requires an increase of the other partner to balance the now greater complement of the first partner: the net result of the rhythmic change in external conditions is thus an increase in both.

In the original hypothesis the nucleic acid was visualized as holding together the long polypeptide chains. Since we now feel impelled to picture the protein component in the form of closed envelopes, the question arises as to how the nucleic acids can be associated. Apparently there is no difficulty about this. In fact a ring structure for nucleic acid containing 4 nucleotides has been suggested by Makino (Makino, 1935), and this structure shows that it is possible to visualize large numbers of interlinked nucleotides (forming perhaps nucleic acids of high molecular weight) stabilizing a single atomic envelope carrying basic *R* groups.

The idea that an association of protein and nucleic acid lies at the heart of the reproduction of specific patterns is of special interest in connexion with those virus proteins which have a nucleic acid component. Specially interesting is the case of the tobacco mosaic virus which contains say 6 % nucleic acid. This substance is found to disintegrate in urea solution with the formation of low molecular weight material which contains no nucleic acid and possesses no virus activity. Specially noteworthy also is the recent demonstration of the great effect of small changes in *pH*, or in the electrolyte concentration, on the rate of disintegration (Stanley & Loring, 1938). In this connexion it is of great interest to notice that Tarnavski (1938) has found that injections of thymonucleic acid induce phenotypic alterations in *Drosophila melanogaster*.

My original analysis drew attention to the possibility that a varying complement of nucleic acid may be an essential element in genic duplication. This suggestion becomes considerably more attractive and illuminating with the fabric theory of protein structure.

In these two respects, the original picture remains qualitatively the same. But there are two important differences. In the original picture, there was no explanation as to how genic units with a chain structure come to possess the highly definite and specific chemical individualities and personalities required of them. Actually, it is the realization that the polypeptide chain hypothesis gives no explanation of the existence of chemical individuals and so is fundamentally inadequate to explain the structure of the enzymes and virus proteins and all the other biologically active proteins which in a sense provides the strongest support for the fabric hypothesis of protein structure. It has already been explained how the idea of an atomic envelope or closed structure provides a possible solution of this puzzle. I therefore now suggest that the picture of the genic unit as an atomic envelope gives to this type of protein unit, as it gives to the enzyme and virus types, a highly definite and specific chemical individuality and personality. This is, if anything, a matter of

greater importance for the gene than for the enzymes and other biologically active proteins.

Secondly, the disturbing of the delicate equilibrium attained by saturating the highly charged protein envelope by an appropriate concentration of acid may, in accordance with our studies on the nature of denaturation, lead to a collapse of the envelope. This collapse is perhaps essentially an opening of the envelope. This suggestion, it seems to me, throws light on the capacity of the genic units to duplicate themselves. This is perforce regarded as a process of catalysis in which the existing pattern of the genic unit facilitates the building of new material into a similar pattern. How this is accomplished is of course not known, but it seems difficult if not impossible to visualize this process except with an open two-dimensional template. A three-dimensional structure can hardly impose its pattern in all its details on new material and a linear pattern with free rotation about bonds lacks that constancy of pattern, which seems to be an essential element in the situation. A closed two-dimensional structure also seems to be excluded.

Now on the fabric theory, it is an outstanding characteristic of globular proteins that instability may lead to a tearing of the fabric. Thus once the balance of protein and nucleic acid is destroyed, either by a change in pH or in some other way, the high charge on the protein leads presumably to the ripping of the closed fabric into an open fabric. This I suggest is the template which leads to the formation of a new fabric in which each element can be superposed on like elements in a manner impossible with a three-dimensional structure or even with a closed two-dimensional structure. It is an essential point in the picture of the protein units as closed surface structures that only the correct complements of residues can form such structures. In this case the formation of new atomic envelopes can presumably be accomplished on the pattern of the existing envelopes, just because the newly formed fabric repeats the pattern of the old. This picture, speculative as it necessarily is, brings the problem of genic duplication into very direct relation with problems of protein chemistry in general, problems which can be studied under less difficult experimental conditions than those within the living nucleus. The part played by ions in stabilizing certain proteins in solution is well known. I suggest that in essence the nucleic acid component in the resting nucleus is playing a very similar role. It is further well known that some types of denaturation are reversible and it has already been suggested that in such cases, in so far as it is not simply a question of the reversible dissociation of a colony of units themselves having the protein

character, there is a partial breakdown of the closed protein fabrics (Wrinch, 1938 *b, c*). In the case of the genes, it seems necessary to picture the breakdown of the envelopes as of a very mild type and entirely reversible.

An interesting fact about insulin gives point to this suggestion. It is well known that protein monolayers form spontaneously from protein in solution and that in many cases they are capable of forming fibrous structures if they are compressed preferentially in any one direction. Presumably the envelopes of the proteins in solution in such cases break down mainly into certain complements of linear peptides, probably peptide rings, which on being compressed in one direction, line up in parallel formation. The case of insulin, however, is different. So far it has not proved possible to form a fibre from insulin monolayers in this way. The conclusion I draw from this fact is that the atomic envelopes in this case, which fragment under the influence of the surface forces, fragment only into fragments which still have a fabric character, or possibly if they fragment into rings, that these rings are rather small, containing perhaps only two or six residues, giving in either case components which do not lend themselves to being aligned in parallel array. In view of the high leucine content of insulin, there seems something in favour of the former alternative and I suggest that it is likely that in this case portions of the fabric may persist intact, even when the envelopes are punctured. It must be remembered that the surface forces are of a very mild type in comparison with those brought into play in denaturation by the ordinary methods. Now in the case of the gene, the very delicately poised equilibrium of highly charged protamine envelopes and nucleic acid may be disturbed by an extremely slight change in external conditions and sufficient amounts of nucleic acid may remain associated with the basic *R* groups to permit the envelope, though ripped open, to remain in one piece, thereby facilitating the reformation in due course of the original atomic envelope. So much attention has been devoted in the last years to denaturation of proteins by means of fierce techniques that it has come to be assumed by many writers that any collapse of the native proteins involves a breakdown of all its "fine structure", so that the residues in "denatured" proteins are linked simply and solely into linear arrays. There is, however, no experimental justification for this assumption. It is for this reason that studies of the structure of protein monolayers are of such great importance. In such cases the prodigious insolubility of the monolayers obtained from highly soluble proteins shows beyond question that some essential characteristic of the structure in solution has been

disturbed (Langmuir, 1938 *a*), yet on the other hand the exciting causes, namely the surface forces, are very mild. Presumably the milder the disturbing agent the greater the chance of our studies revealing some information as to the nature of reversible denaturation. The fact that gene duplication maintains unchanged the chemical personality of the genic unit suggests to me that the disturbing agent may be of an exceedingly mild nature. If this is so there is an enormous interest for cytogenetics in those studies in which, as possibly in the case of insulin monolayers, the domestic architecture of the native molecules is not completely destroyed.

It is also interesting to see the implications for cytogenetics of the supposition that in protein breakdown under definite conditions, e.g. insulin in solution at a certain *pH*, fragmentation takes its own preferred paths, determined by the nature and arrangement of the specific complement of the various residues (Wrinch, 1938 *b, c*; Wrinch & Langmuir, 1939). This supposition was made because it was required by the interesting new facts regarding the differing types of monolayer formed by specific proteins in solution. Thus it is a striking result of this monolayer technique that the films formed by various specific proteins, such as pepsinogen, trypsin and wheat gliadin, can be distinguished in the simplest possible way by means of the expansion patterns formed by puncturing the monolayers with oil (Schaefer, 1938). Still more remarkable are the results with pepsin which is pretreated in different ways. Thus the monolayers formed from pepsin and from pepsin which has been shaken 400 times a minute for 1, 2, 5 or 10 min. can be distinguished by their expansion patterns. Apparently there are differences in the structure of the monolayers formed by this single protein under these different circumstances. This simple and beautiful experiment shows beyond doubt in my opinion that there is a gradation in the degrees of breakdown of the essential globular structure and that any picture of all such monolayers as long-chain peptides indifferently is too naïve and simple to stand up to examination. In any case I would suggest that one of the major themes of protein chemistry which is of very direct relevance to genic structure, in particular to genic reproduction, is the nature of very mild types of protein breakdown, and that the study of such phenomena by means of surface technique offers at the moment the best chance of new knowledge; also that any studies of reversible breakdown are also likely to be illuminating.

To sum up: It seems as yet rather too soon to expect direct chemical data relating to the structure of the genic units; nevertheless there seems on the whole to be a strong case for attributing to some nucleic acid pro-

tein complex the leading role in duplication. The leading part played by the genic units in directing development, as well as in the process of duplication, suggests that they must be chemical individuals with the highest possible degree of specificity. This confronts cytogeneticists with the same puzzle as confronts the student of protein chemistry, who is now forced to regard enzymes, virus proteins and indeed the whole range of biologically active proteins, as chemical individuals. The major puzzle of protein chemistry, namely, the existence of these very large but physically and chemically well-defined molecules, seems impossible to resolve in terms of the polypeptide chain theory of proteins. The fabric theory of protein structure, however, appears to provide a possible solution to this puzzle, by suggesting that the essential entity in such proteins is an atomic fabric which folded round can form atomic envelopes. It is accordingly suggested that the genic protein units are atomic envelopes, and that the nucleic acid plays an essential part of the situation in that the protein components of the genic units are presumably very basic indeed and so need a stabilizing atmosphere of acid if they are to remain stable in the globular form. In accordance with the picture already forced upon us by a consideration of the denaturation phenomena of the biologically active proteins in general, it seems likely that any disturbance of the precarious balance of protein envelope and acid will lead to an open fabric thereby providing the template required for catalysing the formation of new protein units. Data of protein chemistry suggest that such a disturbance can be caused, for example, by a shift of pH , upon which the precise complements of protein and acid previously in balance essentially depends. Thus, as originally suggested, a rhythmic variation in some parameter, e.g. pH , may be the essential agent in genic duplication. Attention is called to the very slight change of pH which may be adequate to this end and it is particularly emphasized that studies in protein chemistry in general, of reversible breakdown of proteins and of the particular breakdown of proteins due to very mild agencies, may be of great importance in elucidating the nature of reproduction in the living organism.

It has also been emphasized that there seems to be a direct relation between the main problems already receiving attention in protein chemistry. In view of this fact it seems important that the closest possible contact should be maintained between the two disciplines. In such a partnership protein chemistry itself also stands to gain considerably, for there can be no doubt that the chromosomes provide for the protein chemist the final challenge.

REFERENCES

- ASTBURY (1938). *C.R. Lab. Carlsberg*, **22**, 45.
BLOCK (1938). *Cold Spr. Harb. Symp.* **6**, 79.
CASPERSON (1936). *Skand. Arch. Physiol.* **73**.
COHN (1938). *Cold Spr. Harb. Symp.* **6**, 8.
HEIDELBERGER (1938). *Cold Spr. Harb. Symp.* **6**, 369.
JORDAN LLOYD (1932). *Biol. Rev.* **7**, 254.
—— (1933). *Biol. Rev.* **8**, 463.
KOSSEL (1928). *The Protamines and Histones*. London.
LANGMUIR (1938 a). *Cold Spr. Harb. Symp.* **6**, 171.
—— (1938 b). *Proc. Roy. Instn.*, 9 December.
—— (1939 a). *Nature, Lond.*, **143**, 280.
—— (1939 b). *Proc. Phys. Soc.* **51**, 592.
LINDERSTROM LANG (1935). *Trans. Faraday Soc.* **31**, 324.
MAKINO (1935). *Hoppe-Seyl. Z.* **232**, 229.
ONCLEY (1938). *Cold Spr. Harb. Symp.* **6**, 19.
REISS (1936). Thesis: "Le pH intérieur cellulaire." Strasbourg.
SCHAEFER (1938). *J. phys. Chem.* **42**, 1089.
SCHIMPER (1881). *Z. Kristallogr.* **5**, 131.
STANLEY & LORING (1938). *Cold Spr. Harb. Symp.* **6**, 341.
SVEDBERG (1939). *Proc. roy. Soc. B*, **127**, 1.
TARNAVSKI (1938). *C.R. Acad. Sci. U.R.S.S.* **20**, 723.
WRINCH (1934). *Nature, Lond.*, **134**, 978.
—— (1935 a). *Nature, Lond.*, **135**, 788.
—— (1935 b). *Nature, Lond.*, **136**, 68.
—— (1936 a). *Protoplasma*, **25**, 550.
—— (1936 b). *Nature, Lond.*, **137**, 411.
—— (1936 c). *Nature, Lond.*, **138**, 241.
—— (1937 a). *Nature, Lond.*, **139**, 972.
—— (1937 b). *Proc. roy. Soc. A*, **160**, 59.
—— (1937 c). *Proc. roy. Soc. A*, **161**, 505.
—— (1937 d). *Int. Congr. Phys., Chem. and Biol., Paris*, p. 395.
—— (1938 a). *Proc. phys. Soc.* **50**, 141.
—— (1938 b). *Phil. Mag.* **25**, 705.
—— (1938 c). *Phil. Mag.* **26**, 313.
—— (1938 d). *Cold Spr. Harb. Symp.* **6**, 122.
—— (1939 a). *Nature, Lond.*, **143**, 482, 763.
—— (1939 b). *Cold Spr. Harb. Symp.* **7**.
WRINCH & LANGMUIR (1939). *Nature, Lond.*, **143**, 49.
WRINCH & JORDAN LLOYD (1936). *Nature, Lond.*, **138**, 758.

THE EQUIVALENT EFFECT OF X-RAYS OF DIFFERENT WAVE-LENGTH ON THE CHROMOSOMES OF *TRADESCANTIA*

BY A. C. FABERGÉ

The Galton Laboratory, University College, London

INTRODUCTION

It is well established that lethals are produced in *Drosophila melanogaster* by high-frequency radiation at a rate which is independent of the wave-length, and proportional to the ionization (Pickhan, 1935; Timoféeff-Ressovsky & Zimmer, 1935; Wilhelmy *et al.* 1936; Fricke & Demereç, 1937). Data on plants are much less extensive (Stadler (1930) on barley, and maize, Stubbe (1938) on *Antirrhinum*). In the case of *Drosophila* radiations investigated range from very soft X-rays of an equivalent wave-length of 2.2 Å. to gamma-rays of 0.02 Å. The importance of this independence of the mutation rate from wave-length has been discussed by the authors mentioned above, and particularly by Timoféeff-Ressovsky *et al.* (1935) and by Stubbe (1938).

Muller, in work not fully published (reported at the Seventh International Genetics Congress, 1939), has shown that 50 kV. X-rays and gamma-rays produce translocations in *D. melanogaster* at the same rate proportionally to the ionization. Such a conclusion was already made likely by the results on lethals, since an appreciable part at least of them are in fact structural changes. Finally, Sax (1940), in a paper published when the present work was already completed, mentions some unpublished results of Rick, showing that structural alterations in *Tradescantia* are produced independently of the wave-length.

It was thought worth while to investigate the chromosome break-producing capacity of X-rays of different wave-lengths in *Tradescantia*. The author has shown that this material lends itself to accurate quantitative work provided an adequate technique is followed.

EXPERIMENTAL TECHNIQUE

The purpose of the experiment was to produce chromosome fragments by the same dose (1500 r.) at two different wave-lengths, keeping other factors known to affect the score as constant as possible. The nature of the discrepancies in this kind of work was discussed in the

380 *Effect of X-rays on the Chromosomes of Tradescantia*

previous paper. In particular it was shown that the variance between the means of slides is very much greater than can be accounted for by the variance between cells within slides. Slides can be used as replicates for getting the experimental error, provided X-ray dosages and other factors can be measured and kept constant with negligible discrepancies. This plan was adopted as it was considered impracticable to repeat the whole experiment a sufficient number of times to base an error term on such replications. Nevertheless, each of the two treatments was carried out twice, so as to check the above assumptions as to reproducibility of conditions.

The fact that the variance between slides is much greater than that within slides has another consequence, that the amount of information about the mean of a group of slides can only be increased to a limited extent by counting more cells in the same slides. Consequently scoring more cells per slide than a certain number may no longer be economical. In the data of this experiment, the 18 cells per slide scored give 79% of the information that would have been available if an indefinitely great number of cells was used from each slide.

A clone of *Tradescantia bracteata* ($n=6$ chromosomes) growing out of doors was used; inflorescences were picked off for irradiation, and afterwards kept in water for 72 hr. at room temperature ($\pm 20^\circ$ C.) before smearing. Permanent preparations stained with gentian violet were used. Cells were drawn at a magnification of 920, those first coming into the field of the oil-immersion lens being chosen. Chromosome pieces were counted in the drawings. There are several advantages in this simple and at first sight even wasteful method of scoring. It is important for the interpretation of this kind of work to know *exactly what* has been scored. It is impossible, for instance, to score all translocations, and it can never be known what proportion has been detected, a proportion likely to vary with the intensity of the effect. The simple counting of pieces is so objective that few cells at the right stage have to be discarded, thus minimizing the possibility of bias in selection.

Cut inflorescences were immersed in a layer of water 1 cm. deep at the bottom of a celluloid dish; they were pressed into that layer by threads stretched across the surface of the water. The dish was placed on a big block of paraffin wax. Eleven branches were used on each occasion. The purpose of immersion in water, which was also used by Sax (1940), is twofold. First, it minimizes errors of dosage measurement due to scatter of X-rays by the material, water probably having absorbing and scattering properties very similar to plant tissue. Secondly, it makes

it easier to keep the material at constant temperature, which is necessary as a higher temperature is very effective in promoting the rejoining of broken chromosome ends. Inflorescences were placed in the water at 22° C. 10 min. before treatment, which is known to give ample time to bring them very close to the temperature of the water.

The two radiations used were:

Soft radiation. 50 kV. Constant potential circuit; 8 mA.; W target; radiation unfiltered, except for glass of tube, etc. Half-value layer = 1.85 mm. Al. Equivalent wave-length = 0.44 Å.

Hard radiation. 400 kV. peak, pulsating high potential "Victor" tube; 3 mA.; W target; filters: 0.22 mm. Sn, 1.7 mm. Al, and inherent filtration equivalent to 2 mm. Cu. Equivalent wave-length = 0.077 Å.

Dosage was determined with a "Victoreen" dosimeter, the thimble ionization chamber being placed at the top and bottom of a paraffin wax layer of the same thickness, and occupying the position of the layer of water and material. The measuring instruments of the X-ray apparatus³ were kept at the same constant values during treatment.

For the softer radiation, the material was placed at 20 cm. from the target; the mean dosage rate over the 1 cm. layer was 29.09 r. per minute, and so the exposure given was 51.7 min. for 1500 r. It was found that the same dosage rate could be obtained at 52.2 cm. for the higher voltage tube, and the material was placed at the focal distance.

EXPERIMENTAL DATA

Table I gives the frequencies of cells with various numbers of chromosome bodies scored in this work. A and B are the two repetitions at the softer radiation, C and D at the harder. It was originally planned to use five slides in each group; unfortunately only three satisfactory slides were obtained in batch A, as owing to the lateness of the season the inflorescences were rather old. The mean number of chromosome bodies per cell is given at the right of the table.

An analysis of variance on these data is given in Table II. It will be seen that the repetition at the softer wave-length gives a mean square which is not significant, while the repetition at the hard radiation gives a mean square less than that for error. This shows that the technique was satisfactory, and that the assumption made as to the reproducibility of the dose and other conditions is well founded.

The difference between the hard and soft radiations gives a mean square which is less than the error. To express quantitatively the precision of the experiment, we may calculate the 5% fiducial limit, a

382 *Effect of X-rays on the Chromosomes of Tradescantia*

difference between means of 0.867 fragment per cell. That is, if the true difference between the samples were in fact 0.867, either positive or negative, there would be 1 chance in 20 or less of obtaining the small difference actually observed of 0.1097 fragments per cell.

TABLE I

Experimental data

	No. of chromosome fragments in cell											Total	Mean per cell
	6	7	8	9	10	11	12	13	14	15	16		
A	2	2	4	2	3	2	2	.	1	.	.	166	8.2708 λ_c = 0.44 A.
	2	4	6	1	2	2	.	1	.	.	.	152	
	.	4	4	4	2	2	1	.	.	.	1	166	
B	.	4	2	5	1	3	2	1	.	.	.	169	
	6	7	3	1	1	128	
	4	5	7	1	1	134	
	2	8	2	3	2	1	142	
	4	5	7	1	1	134	
C	.	1	5	6	1	2	2	1	.	.	.	170	8.1611 λ_c = 0.077 A.
	2	3	4	3	1	3	1	1	.	.	.	160	
	8	4	4	.	.	1	1	131	
	5	8	3	.	2	130	
	1	7	2	1	4	1	2	155	
D	5	3	3	5	.	1	1	143	
	3	9	4	2	131	
	5	5	2	6	135	
	3	5	3	4	1	1	1	146	
	1	1	4	3	4	4	1	168	
	53	85	69	48	26	23	14	4	1	0	1	2660	

TABLE II

Analysis of variance

	D.F.	Sum of squares	Mean square	Variance ratio	5 % variance ratio
Wave-length	1	17.3361	—	(13.6)	—
Repetition in wave-length 1	1	745.0083	—	3.17	4.60
Repetition in wave-length 2	1	52.9000	—	(4.45)	—
Error	14	3293.8667	235.276	—	—
Total	17	4109.1111	—	—	—

DISCUSSION

Apart from their general interest in connexion with the relation between mutation and chromosome breakage, the results of this experiment may have a bearing on another matter, special to this material. *Tradescantia* chromosomes behave differently when X-rayed than do those of *Drosophila*. The author (Fabergé, 1940) interpreted his experi-

ments, in the sense that an increasing X-ray dosage has two effects: it increases the frequency of breaks, and also interferes with the rejoining of broken ends. The author is inclined to the view that the second effect is a direct result of the first, for instance that chromosomes broken in several places get out of alignment more easily. Though plausible, such a view is by no means proved. The possibility exists that there are two *separate* effects of X-rays on *Tradescantia*, the first of which only, that of producing breaks, is represented in *Drosophila*. That undefined physiological conditions can determine whether broken chromosome ends will repair themselves in different kinds of plant tissue has been shown by McClintock (1939). A significant difference between the means of the two treatments would have meant that either one or the other of these two effects is not independent of the wave-length. The formal possibility remains that both effects depend on wave-length, but so as to compensate one another; this seems so unlikely as not to require consideration.

There is little to discuss except to record the purely empirical agreement of these results with other mutation data. Granted that chromosomes are broken fairly directly by ionizations, the data show that the sensitive structures are of a size not to be affected by the differences in the spatial distribution of ions produced by the two radiations. It so happens that with the two wave-lengths used this difference is not very great, and its estimation subject to many uncertainties. The beams used are not monochromatic, and their components are not symmetrically distributed about the equivalent wave-length. In the regions concerned the proportion of Compton scattering increases sharply with the wave-length, and also depends on the nature of the irradiated material.

SUMMARY

An experiment is described in which the number of chromosome fragments produced in *Tradescantia* pollen grain divisions by a dose of 1500 r. of two radiations of λ_e 0.44 Å. and λ_e 0.077 Å. is compared. The mean numbers of chromosome bodies per cell are 8.27 and 8.16 respectively. The difference, 0.11, is less than its own standard error of 0.44.

I am very much indebted to Mr J. R. Clarkson for carrying out the irradiations and measurements in the Physics Department of the Royal Cancer Hospital, London.

384 *Effect of X-rays on the Chromosomes of Tradescantia*

REFERENCES

- FABERGÉ, A. C. (1940). "An experiment on chromosome fragmentation in *Tradescantia* by X-rays." *J. Genet.* **39**, 229-48.
- FISHER, R. A. (1936). *Statistical Methods for Scientific Research Workers*, 6th ed. London and Edinburgh.
- FRICKE, H. & DEMEREÇ, M. (1937). "The influence of wave-length on the genetic effects of X-rays." *Proc. nat. Acad. Sci., Wash.*, **23**, 320-7.
- GOODSPEED, T. H. & UBER, F. M. (1939). "Radiation and plant cytogenetics." *Bot. Rev.* **5**, 1-48.
- MCCLINTOCK, B. (1939). "The behaviour in successive nuclear divisions of a chromosome broken at meiosis." *Proc. nat. Acad. Sci., Wash.*, **25**, 405-16.
- MAYNEORD, W. V. (1934). "The physical basis of the biological effects of high-voltage radiations." *Proc. roy. Soc. A*, **146**, 861-79.
- PICKHAN, A. (1935). "Vergleich der mutationsauslösenden Wirkung von Röntgen- und Gammastrahlen bei *Drosophila melanogaster*." *Strahlentherapie*, **52**, 369-88.
- SAX, K. (1940). "An analysis of X-ray induced chromosomal aberrations in *Tradescantia*." *Genetics*, **25**, 41-68.
- STADLER, L. J. (1930). "Some genetic effects of X-rays in plants." *J. Hered.* **21**, 3-19.
- STUBBE, H. (1938). "Genmutation. 1." *Handb. der Vererbungswiss.* p. 23. Berlin.
- TIMOFÉEFF-RESSOVSKY, N. W. & ZIMMER, K. G. (1935). "Wellenlängeunabhängigkeit der mutationsauslösenden Wirkung der Röntgen- und Gammastrahlen bei *Drosophila melanogaster*." *Strahlentherapie*, **54**, 265-78.
- TIMOFÉEFF-RESSOVSKY, N. W., ZIMMER, K. G. & DELBRÜCK, M. (1935). "Über die Natur der Genomutation und der Genstruktur." *Nachr. Ges. Wiss. Göttingen, Fachgr. 6, N.F.* **1**, 190-245.
- WILHELMY, E., ZIMMER, K. G. & TIMOFÉEFF-RESSOVSKY, N. W. (1936). "Einige strahlen genetische Versuche mit sehr weichen Röntgenstrahlen an *Drosophila melanogaster*." *Strahlentherapie*, **57**, 521-31.

THE SIGNIFICANCE OF DIPLOIDITY AND CROSSING-OVER (THEORY OF DIFFERENTIAL PERIODICITY)

By G. F. SLEGGs

(With Five Text-figures)

INTRODUCTION

THE present writer has elsewhere (Sleggs, 1939) advanced the theory that differentiation is due to the direct interaction of genes, which are arranged along the chromosome at various angles of rotational stagger.

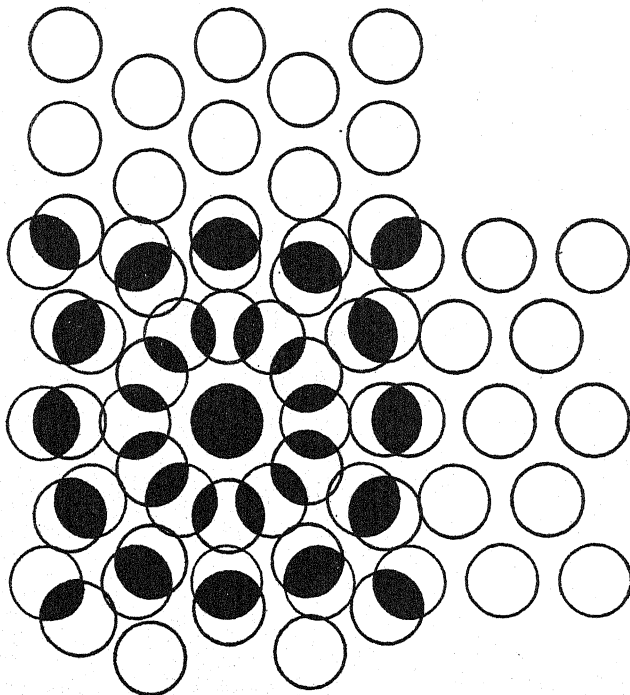


Fig. 1. Schematic morphogenetic field, in the form of floral pattern, formed by staggering two lattices whose units are represented by circles. By blackening overlap of units variations in chemical strain, and hence synthetic activity, are shown, to which the growth pattern will correspond. Note inequality in number of parts, and alternation of whorls. If the pattern be extended by superposing a greater area of the lattices, the portion shown is repeated as in an inflorescence.

The repeated formation of new nuclei is equivalent to the spreading of chemical lattices in superimposition, which produces pattern on the principle under which gauzes produce an optical pattern when so

386 *The Significance of Diploidity and Crossing-over*

staggered. The considerations upon which this deduction is founded are:
(1) a number of fundamental morphogenetic pattern factors—radial and

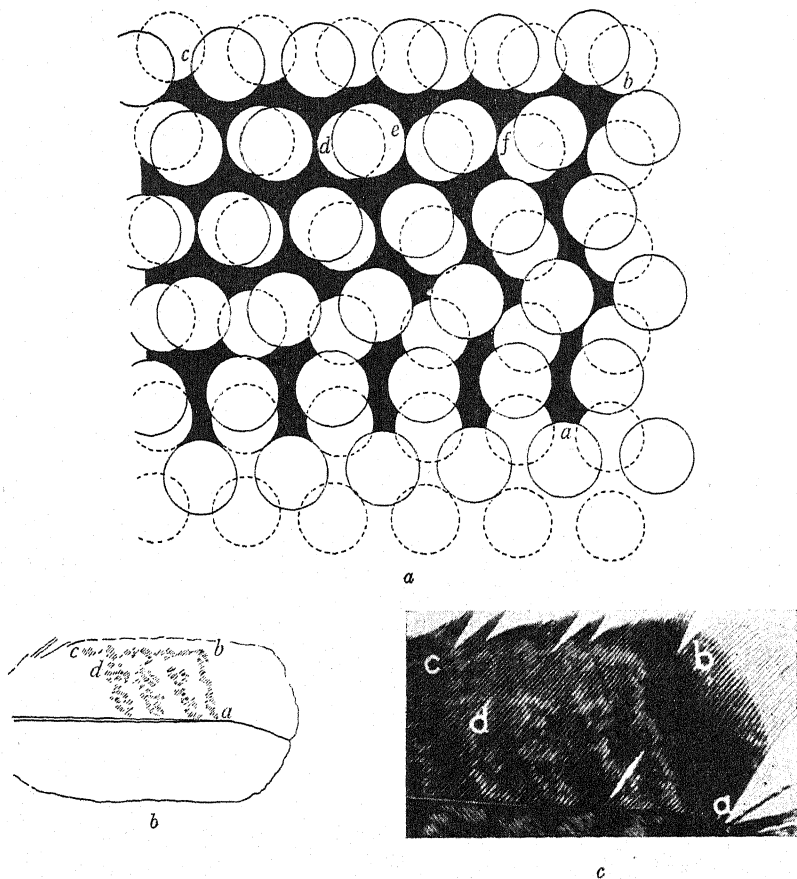


Fig. 2.

bilateral symmetry, annularity, concentric whorling of structures differing in character and number, as in floral pattern—can be interpreted mathematically in terms of the spatial periodicity differential arising from rotational gene stagger¹ (Sleggs, 1940) (Fig. 1); (2) the hypothesis

¹ *Example of morphological evidence.* By superposing two lattices (Fig. 2a), each consisting of equally spaced circles (the arrangement being triangulated in one case, quadrangulated in the other) at a rotational angle of 7° , and plotting the morphogenetic field so generated (by blackening the portions not occupied by circles), an approximation is obtained to the pigmentation pattern of the shown feather (turkey, b, c). The pattern comprises repeated broken zigzag stripes, the end one, ab, sweeping back longitudinally (bc), with salient regions (e, f) opposite the transverse stripes. The 4th of such regions (d) joins the adjacent transverse stripe, as in the feather.

that morphological pattern is a compound differential periodic wave-field can explain the evolutionary problem of the addition of organs without substantial disturbance of previous pattern—since slight changes in differential wave-lengths can produce summing of morphogenetic gradient crests leading to intense local specialization with slight modifications elsewhere (Sleggs, 1940); (3) chemical lattices extending in rotational stagger constitute a harmonic equipotential system, furnishing the basis for regeneration and regulation sought by Driesch (and still theoretically of interest notwithstanding attempts (Dalcq, 1938) to discredit Driesch's conception as subjective); (4) the units of such lattices are staggered in rising and falling gradients, explaining repetition of parts, which cannot be accounted for by cytoplasmic gradients; (5) the horizontality of such lattice extension interprets the tendency to membranous growth; (6) to derive differentiation from the direct interaction of genes interprets linearity of genes; (7) the fact that genes must be arranged in a column and yet the column can be broken (into chromosomes) is intelligible on the view that the angle of rotation between certain adjacent genes has the value zero, implying no contribution to differentiation and no need for intactness at this point in the column; (8) giant chromosomes appear to be clusters of normal chromosomes having differential stagger patterns (Metz & Lawrence, 1937; Dobzhansky & Scolov, 1939); (9) the hypothesis of rotational stagger accords with observable zigzag (Belling, 1926) and spiral (Darlington, 1932) chromosome formation.

GENE COLUMN

It has been maintained (see Gulick, 1938) that the genes cannot be regarded as stacked in the chromosome like coins in a pile, since such a condition would involve electrical polarity of the chromosome, which is not actually observed. But this contention appears to leave out of account the energy relations which may arise between the edge of the chromosome and its surroundings, whereby the electric potential will be converted into synthetic activity. The present theory derives the process of differentiated growth from such synthetic activity. The essential nature of the gene column in its functional phase is shown in Fig. 3, the genes being represented schematically as small quadrangular units (quadrangularity being best adapted to show rotational stagger). The intergene substance is a region of chemical strain (or tilted valencies, owing to structural relations with staggered genes), such strain leading to the intense synthetic activity which is characteristic of living matter. The morphological pattern determined by the gene column is represented

in the angles of rotation at which the genes are relatively orientated, a periodic component being contributed by the interaction between each two adjacent genes. The system is self regulative in that restagging of

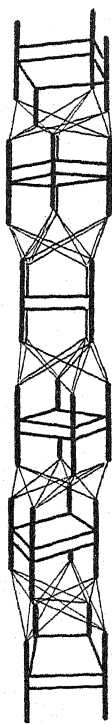


Fig. 3.

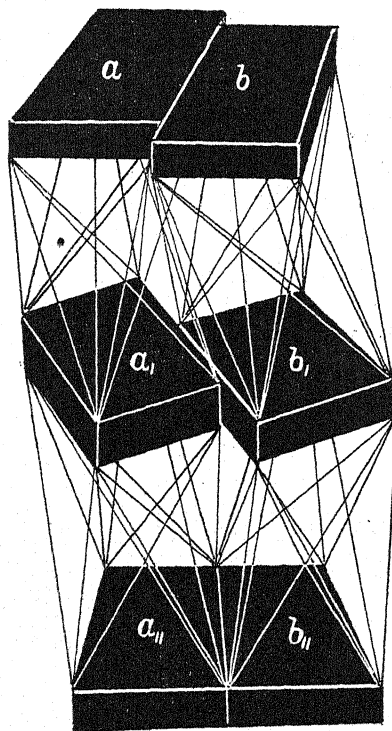


Fig. 4.

Fig. 3. Diagram of gene column, with six genes in rotational stagger. Thin connecting lines are a formal representation of chemical strain, which will vary along a line of chromosomes derived from this by lateral extension, since rotational stagger becomes combined with lateral, in respect of recurring genar points.

Fig. 4. Synopsis of gene columns, each having three genes. Differences in rotational angles, or in lateral stagger, will intensify chemical strain, depicted by thin connecting lines.

the gene column (under the influence of organizing or evocating substances) can transform the field into one of different prospective potency.

DIPLOIDITY

Since the form of the gene column is related to the morphological pattern which it generates, columns from morphologically different individuals will differ in the angles of gene stagger. Between the members of a species such stagger-pattern differences will be slight, allowing the columns to pair laterally in synopsis (Fig. 4) but with an increase in

chemical strain, since one distorts the other slightly. This results in intensified synthetic activity, accounting for the fact that fertilization is followed by a sharp increase in oxidation and henceforth in a course of active growth, whereas the synthetic powers of the haploid system are so feeble that as a rule it fails to develop at all. Thus the theory can assign a direct and fundamental function to sexuality. If the determinators (gene columns) are from the same individual and alike, synapsis produces no increase in strain or synthetic power, accounting for Morgan's observation (1926) that artificial restoration of diploidity produces no improvement in a weak haploid individual.

CHIASMATYPY

The pairing, or chemical union, of determinators of different pattern is equivalent to the union of already twisted threads, which will lead to intensified twisting of the double column, but such chiasmotypy can only replace the added chemical strain to a fractional extent except where the difference in pattern happens to be uniformly graded lengthwise.

DOMINANCE

If determinators, on the whole, match each other fairly closely, but differ markedly in the angles of certain pairs of genes, the latter will pair like springs applied in a state of tension. The more strongly installed will suffer slight change, the weaker sustaining pronounced alteration.

BLENDING

The theory thus deduces that one character will predominate over another, but that degrees of dominance will vary, intelligible on the comparison of the interaction of springs of different strengths. In any event the morphological patterns will not be commingled like separate patterns printed one over the other on a sheet of paper, but will have their contrasting elements combined into single elements from the fact that in synapsis the two determinators unite, without break, to form one.

LETHALITY OF GENES

A marked rotational difference between two pairing genes may lead, sooner or later, to breakdown of synapsis, a breach in the double column weakening or destroying its power to synthesize.

CROSSING-OVER

Under certain conditions the tension arising in the union of determinators can be relieved by an exchange of opposing portions of the two columns. The principle involved is illustrated in Fig. 5, in which difference in sinuosity is allowed to represent difference in angulation pattern. The

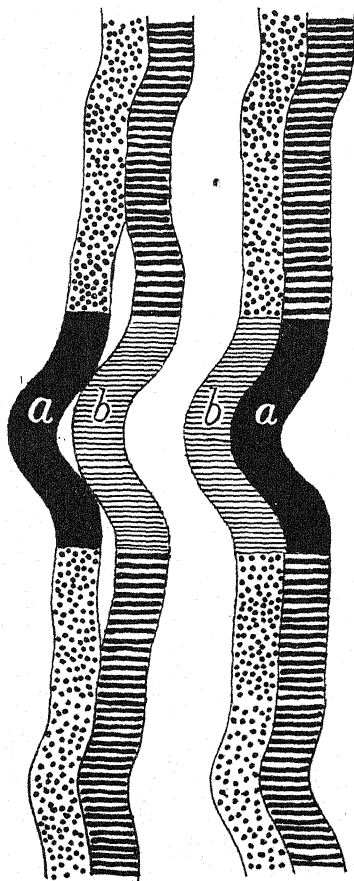


Fig. 5. Showing essential nature of crossing-over. Exchange of portions *a*, *b* enables columns to pair through reduced divergence in pattern.

exchange of the portions *a* and *b* lessens the difference between the homologous determinators. Transcribed morphologically, divergent structural elements of individuals are, in maturation, so shuffled as to reduce difference in the pattern as a whole. Hence the species tends to maintain structural uniformity. The theory thus integrates specificity and sexual potency.

ORIGIN OF SPECIES

Where wide structural diversification arises within a species, the portions due for crossing over may become so extensive that the remaining untable portions represent a degree of union insufficient to determine the act of crossing over. The determinators even at fertilization will increasingly fail to pair, leading to increasing infertility and eventual scission of the species into two or more groups sufficiently close structurally to reproduce. These, henceforth, will continue as separate species.

DISCUSSION

It would seem obvious that life did not come into existence in search of energy, but was driven into existence by the flow of solar energy. Life thus represents the flow of energy through a tortuous and enlarged channel, the tortuosity being represented by chemical strain, and the enlargement by the increased absorption of energy in the synthetic activity resulting from such strain.

Thus, both tortuosity and enlargement are maintained and extended by the flow itself, the evolutionary extension (which takes the form of increasing gene stagger and differentiation) probably arising from the effect of disturbing extraneous factors, in the way that an obstacle can increase the meandering of a stream. But in this case the channel lies through an architectural system of accurately constructed and recurring units forming a chemical cagework, which, in differentiation, grasps more energy the more it is distorted. Hence, chemical energy increases as differentiation advances (Needham, 1927), up to a point, presumably, where the energy potential is unable to drive the process any further forward; and here growth comes to an end. Stated in terms of the gene column, positions arise in the spreading process of the genar lattices where the degree of chemical strain calls for a higher energy potential than is available.

SUMMARY

Deductive evidence is presented, from nine sources, for the view that differentiation proceeds from the direct interaction of rotationally staggered genes. Such staggering produces chemical strain and hence the high synthetic activity of living matter. Morphological pattern is a compound differential periodic wave field, the components of which are determined by the angles of rotation between successive genes. Hence the form of the chromosome is related to the pattern it determines. Sexual interaction involves the chemical union of gene columns of

392 *The Significance of Diploidity and Crossing-over*

different form, intensifying the chemical strain and leading to the increased power of synthesis (growth) possessed by the diploid system. Dominance results from the interaction between a strong portion of the chemical cagework of one chromosome and its weak counterpart, which yields like a weak spring pulled by a strong, the resulting double gene having approximately the rotational stagger of the stronger. Gene columns derived from markedly dissimilar morphological sources will, owing to corresponding dissimilarity in stagger pattern, have difficulty in pairing, but by interchange of portions at maturation, this dissimilarity can be reduced. Hence crossing-over reduces individual divergence within the species. If divergency nevertheless would involve crossing over of great lengths of chromosome such interchange may become mechanically impossible, leading to the eventual impossibility of pairing at fertilization, and hence infertility. From this the origin of new species (from structurally close races of the parent species) is deduced.

REFERENCES

- BELLING, J. (1926). Quoted (re zigzag chromosomes) by Darlington.
 DALCQ, A. M. (1938). *Form and Causality in Early Development*. Cambridge.
 DARLINGTON, C. D. (1932). *Recent Advances in Cytology*. London.
 DOBZHANSKY, TH. & SCOLOV, D. (1939). *J. Hered.* **30**, 1, 3-18.
 GULICK, A. (1938). "What are the Genes?" *Quart. Rev. Biol.* **12**, 2.
 METZ, C. W. & LAWRENCE, G. E. (1937). *Quart. Rev. Biol.* **12**, 2.
 MORGAN, T. H. (1926). *The Theory of the Gene*. New Haven.
 NEEDHAM, J. (1927). *Chemical Embryology*. Cambridge.
 SLEGGS, G. F. (1939). "The theory of differential periodicity." *Growth*, **3**, 173-9.
 — (1940). "Morphogenetic pattern elements according to the theory of differential periodicity." *Growth*, **4**, 1.
 — (1940). "The processes of growth according to the theory of differential periodicity." *Growth*, **4**, 1.

THE INFLUENCE OF NUTRITION UPON POLLEN GRAIN SIZE IN *LYTHRUM SALICARIA*

By HELEN SCHOCH-BODMER

St Gall, Switzerland

(With Three Text-figures)

As a rule, pollen grain size is regarded as a character that is not influenced by exterior factors. Piech (1922), however, was able to show that in some species, as, for instance, *Linaria genistifolia* and *Antirrhinum maius*, the pollen grains are subject to the influence of temperature, low temperature having a tendency to reduce their size. Later on, Krumbholz (1926) found a similar reaction in some species of *Oenothera*, the pollen grain size of which decreases towards the end of October, equally as a consequence of a change in exterior factors.

Lythrum Salicaria L., a heterostylous species, is known to form three types of pollen grains, long, mid and short ones. It was Tischler (1917, 1918), who advanced the hypothesis that the different types of pollen grains in heterostylous species might be due to differences in the supply of nutritive substance to the three types of stamens. The investigations of the present writer have shown that there is within each of the three types of *Lythrum* pollen a wide range of variation (1927, 1937b, 1938b) and even a transgression of pollen sizes between the minima of the long pollen type and the maxima of the mid-pollen type, the same being true for mid and short pollen. These findings seem to support the theory that nutrition plays an important part in the determination of pollen size in *L. Salicaria*. Another fact in favour of Tischler's hypothesis is the occurrence of mid or transitional stamens in mid-styled flowers. In certain mid-styled plants single flowers are found with one or two (rarely three or four) mid-stamens in the place of short or long stamens. Besides these there are transitional stages between short and mid-stamens as well as between mid and long stamens. As a rule, but not regularly, pollen size increases with the length of the stamens. The occurrence of these transitional forms shows that primordia for long or short stamens can be changed into the transitional stages under local nutritive influences. Mid or transitional stamens in the place of short ones were chiefly found in the first flowers of vigorous mid-styled plants, cultivated in a rich soil soaked with water. Mid-stamens instead of long ones, on the other hand,

are observed in mid-styled plants (or parts of clones) that grow under less favourable conditions (dry soil). There is, moreover, a third type of mid-stamen in mid-styled plants, where a long and a short stamen are fused to a mid one, frequently with a broad filament and two anthers containing two types of transitional pollen (see Schoch-Bodmer, 1938*b*, p. 84 and Table 12).

The problem of pollen size is, of course, not only a *physiological* one, but a *genetical* one as well. Any observer can immediately indicate whether a plant is a long-, mid- or short-styled one, and whether the stamens are long, mid or short or of a transitional order. But within each type the stamen length and pollen size is of great variability and can be changed by nutrition.

The experiments were carried out with clones; each plant was divided into two or three parts, of which one was cultivated in dry soil in a wooden tub on a balcony, the other in a garden bed with moist soil, and the third in a barrel that was sunk into a garden bed and the soil of which was constantly soaked with water. The soil was the same for all three parts of the clone. The flowers were taken either from main (vigorous) or from lateral (weak) inflorescences and were either main (central) flowers of dichasiums or lateral ones. The pollen was dried on a slide exposed to sunshine and then immediately embedded in Canada balsam, a method that has been described by the present author (1927) and later on by Ferguson & Coolidge (1932). The dry pollen grains only contain traces of water and can be kept for several years without changing their shape and size (Bodmer, 1927; Schoch-Bodmer, 1938*b*). The appearance of these pollen grains embedded in Canada balsam is exactly that of air-dry pollen grains. In water or glycerine the grains round up and change their size after a short time, the plasmatic membrane becoming permeable and the water-soluble substances escaping through the cell wall. The diameter of the grains therefore diminishes considerably, due to the loss of turgor pressure. Hence it does not seem advisable to measure pollen grains in glycerine or water.

Table 1 shows lengths of stamens and pistils determined at a 17-fold enlargement and indicated in millimetres; the average longitudinal diameter is indicated in microns. The enlargement used is a 385-fold one, and for each average value 100 pollen grains were measured. The quotient of longitudinal to transverse diameter shows that the former is about twice the latter; in "long" pollen the quotient is generally somewhat below, in the other types somewhat above 2. In a third column the coefficient of correlation is indicated. There is invariably a positive

TABLE I
Influence of nutrition upon pollen grain size

No. of plant	Date	Conditions of growth	Long pollen			Mid-pollen			Short pollen		
			Length of long stamens or pistils mm.	Length of mid stamens or pistils mm.	Length of short stamens or pistils mm.	Average longitudinal diameter μ §	Quo- tient $L:T$	Coef- ficient of corre- lation¶	Average longitudinal diameter μ	Quo- tient $L:T$	Coef- ficient of corre- lation
L ₂	29. vi. 38	Moist soil, main inflorescence, main flower	10.6	7.1-7.3	3.7-4.6	—	—	—	—	—	—
	8. viii. 38	Moist soil, lateral inflorescence, lateral flower	8.5	5.5-6.2	2.4-3.9	—	—	—	31.4±0.16	2.18	0.49
S ₁ *	5. viii. 38	Moist soil, main inflorescence, main flower	11.3-13.2	7.6-8.4	4.6	—	—	—	37.8±0.18	2.02	0.6
	5. viii. 38	Moist soil, lateral inflorescence, lateral flower	10.0-10.7	5.9-6.8	2.9	—	—	—	32.8±0.16	2.06	0.4
M ₂ †	4. viii. 38	Soil soaked with water, main flower	11.9-12.7	8.7	3.9-4.6	52.0±0.25	1.92	0.64	—	—	—
	5. viii. 38	Dry soil, main flower	11.6-12.2	8.2	3.0-4.2	51.3±0.19	1.95	0.56	—	—	—
M ₃ ‡	8. viii. 38	Dry soil, lateral flower	9.4-10.1	6.65	2.8-3.6	46.8±0.16	1.93	0.49	—	—	—
	4. viii. 38	Soil soaked with water, main flower	11.0-11.3	7.1	4.2-4.9	51.2±0.17	1.96	0.33	—	—	—
M ₄	30. vi. 38	Moist soil, main flower	9.3-10.2	6.65	3.6-4.5	49.6±0.24	2.07	0.5	—	—	—
	5. viii. 38	Dry soil, lateral flower	7.8- 8.7	5.35	2.8-3.3	45.1±0.19	2.0	0.43	—	—	—
	8. viii. 38	Dry soil, lateral flower	8.65- 9.5	5.8	3.4-3.9	44.6±0.19	2.03	0.53	—	—	—
	18. vii. 38	Moist soil, main flower	10.9-11.3	7.1	3.6-4.5	48.1±0.21	1.81	0.36	—	—	—
	3. viii. 38	Moist soil, lateral flower	9.7-10.5	6.3	2.9-3.4	44.6±0.22	1.9	0.47	—	—	—

* See photomicrograph, Fig. 2.

† See photomicrograph, Fig. 3.

§ Average length of pollen grains and standard error σ/\sqrt{n} , n being always 100.

|| Quotient of longitudinal and transverse diameter (average values).

¶ Coefficient of correlation between longitudinal and transverse diameters of each series of 100 pollen grains.

‡ See photomicrograph, Fig. 1.

396 *Influence of Nutrition upon Pollen Grain Size*

correlation between the two diameters of each series of 100 pollen grains.

In all the five plants examined, pistil and stamen lengths, as well as pollen grain size, are influenced by nutrition. The most remarkable differences in pollen size are found between main and lateral flowers;

TABLE II
*Continuous series of pollen sizes; transgression
of mid- and short-pollen averages*

No. of plant	Length of stamens mm.*	Type of pollen	Average longitudinal diameter of pollen grains (L) μ	Average transverse diameter of pollen grains (T) μ	Quotient L:T
M ₂	12.3	Long	52.2 \pm 0.25†	27.0 \pm 0.19	1.93
M ₃	11.1		51.2 \pm 0.20	26.1 \pm 0.21	1.96
M ₅	12.1		50.9 \pm 0.23	28.1 \pm 0.25	1.82
S ₁	12.2		49.8 \pm 0.25	27.5 \pm 0.25	1.81
M ₂	12.0		49.5 \pm 0.22	27.2 \pm 0.19	1.82
M ₅	11.9		48.1 \pm 0.20	25.7 \pm 0.23	1.87
M ₂	9.8		46.8 \pm 0.16	24.3 \pm 0.17	1.93
M ₃	8.2		45.2 \pm 0.19	22.6 \pm 0.19	2.0
M ₅	9.1		44.7 \pm 0.19	22.0 \pm 0.19	2.03
M ₃	10.3		41.6 \pm 0.18	23.7 \pm 0.20	1.76
M ₅	6.0†	Mid or transitional	41.2 \pm 0.19	21.9 \pm 0.22	1.88
M ₂	8.0		40.4 \pm 0.31	21.8 \pm 0.23	1.85
M ₃	6.3		39.1 \pm 0.18	21.5 \pm 0.18	1.82
M ₂	8.0		38.1 \pm 0.26	20.6 \pm 0.25	1.85
M ₂	7.0		37.8 \pm 0.22	19.9 \pm 0.21	1.90
S ₁	8.0		37.8 \pm 0.18	18.7 \pm 0.17	2.02
L ₃	7.6		37.5 \pm 0.21	17.9 \pm 0.21	2.09
M ₅	7.0		36.6 \pm 0.21	19.1 \pm 0.22	1.92
M ₅	7.1		35.3 \pm 0.23	17.8 \pm 0.21	1.98
M ₂	7.5		34.4 \pm 0.22	17.0 \pm 0.18	2.02
L ₂	7.2		34.3 \pm 0.18	15.6 \pm 0.15	2.20
S ₁	6.3		33.0 \pm 0.18	16.6 \pm 0.21	1.99
M ₃	5.3		32.5 \pm 0.16	15.7 \pm 0.16	2.07
M ₃	5.6		31.6 \pm 0.17	15.9 \pm 0.17	1.98
L ₂	5.8		31.4 \pm 0.16	14.5 \pm 0.16	2.16
M ₅	4.3	Short	34.1 \pm 0.21	17.4 \pm 0.21	1.96
M ₂	4.3		33.9 \pm 0.18	15.5 \pm 0.19	2.19
M ₃	3.5		33.1 \pm 0.21	16.8 \pm 0.16	1.97
M ₅	5.0		32.5 \pm 0.18	16.0 \pm 0.17	2.03
M ₂	4.0		31.5 \pm 0.22	15.6 \pm 0.19	2.02
L ₃	4.5		31.4 \pm 0.19	14.5 \pm 0.18	2.16
M ₅	3.2		30.7 \pm 0.20	14.6 \pm 0.2	2.10
M ₂	3.2		30.8 \pm 0.18	13.7 \pm 0.16	2.25
M ₃	3.6		29.8 \pm 0.17	13.9 \pm 0.20	2.14
L ₂	4.0		29.8 \pm 0.15	13.8 \pm 0.20	2.16

* The stamens were measured from the basis of the pistil to the middle of the anther.

† Standard error σ/\sqrt{n} ; n being always 100.

‡ Flowers with one or two mid-stamens, instead of short or long ones, are frequently found in mid-styled plants. Their pollen covers the same scale of sizes as does the mid-pollen of long- and short-styled plants; in some cases it has been found to be larger.

the moisture of the soil does not play so important a part as the position of the flower on a main, i.e. vigorous, or a lateral (weak) inflorescence. The difference in average pollen size of main and lateral flowers can

exceed $5-6\mu$ in extreme cases. Further data on the influence of nutrition upon pollen size were published in 1938^b (Table 12, pp. 92, 93).

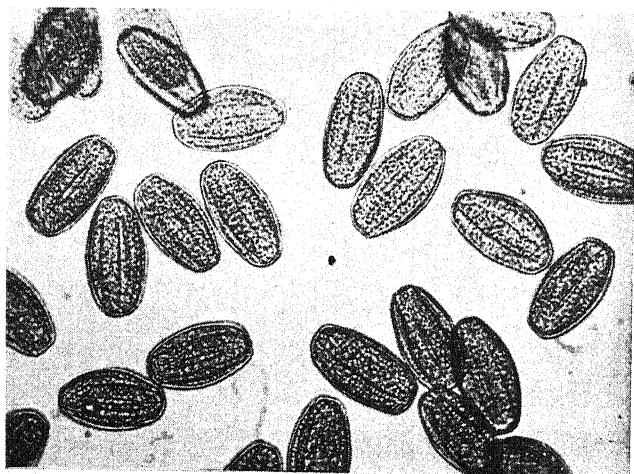
Table II gives a survey of the variability of pollen size found in a number of plants during the course of several years. In this table special stress is laid on the mid and transitional stamens of mid-styled flowers. The correlation between stamen length and pollen size is not a very marked one, though there is a general trend of increase of pollen size with the increase of stamen length.

In connexion with the fact that pollen size can be modified by nutrition, the question is of interest, whether fertility is dependent on pollen size, as it seems to be the case in heterostylous species of *Primula* (see Ernst, 1936). When we examine Tables I and II, it strikes us that mid-pollen of a long-styled or a short-styled plant can be of the same size or even smaller than the short pollen of a mid-styled plant. The fertility of these pollen types is, however, not determined by their size, mid-pollen (of L_2 , for example) being fertile on a mid-styled flower, short pollen of the same size (M_2) fertile on a short-styled flower, while the reciprocal crosses will remain sterile. Genetical reasons are here out of question, since fertility is independent of genetic constitution in *Lythrum Salicaria* (see v. Ubisch, 1925). So there must be differences in the contents of the pollen grains that are not expressed in their exterior shape. Long pollen is full of large starch grains, mid-pollen contains a variable amount of starch grains, while short pollen is mostly free from starch¹ (Tischler, 1917; Bodmer, 1927). It is, however, difficult to realize in which way these storage materials might be an impediment for the penetration of a pollen tube into a style. With a long-styled plant we might assume that the storage material of mid and short pollen is not sufficient to allow the pollen tubes to grow down into the ovary. But it is not clear why tubes of long pollen grains should not regularly penetrate into the ovary of a mid- or short-styled plant. Moreover, fertility does not only depend on the pollen contents, but also on the structure and length of the styles. The problem is therefore a very complicated one. In all experiments on fertility the qualities of the pollen as well as those of the styles must be taken into consideration. Darwin (1877), Barlow (1923), Stout (1923) and East (1927) have shown that long- and mid-styled plants have a tendency to be self-fertile, which is not the case in short-styled plants. Long-styled plants are sometimes fertile with mid-pollen, some of the mid-styled plants with long, others with short pollen. Similar results have been obtained in the present writer's experiments,

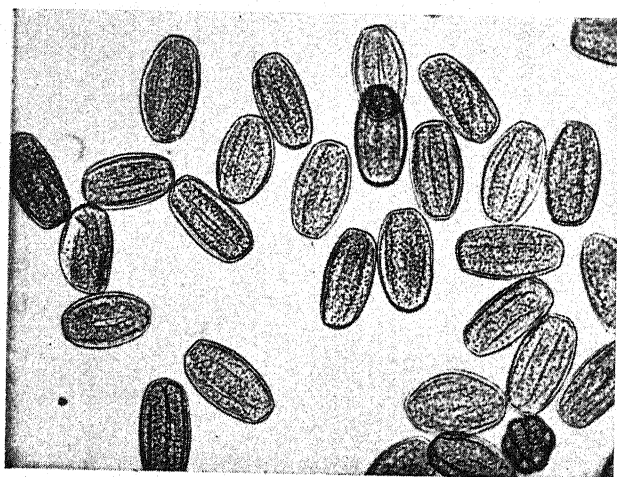
¹ See photomicrographs.

398 *Influence of Nutrition upon Pollen Grain Size*

showing that a reduction in style length brings about self-fertility with short pollen, while mid-styled flowers with styles longer than the average



a

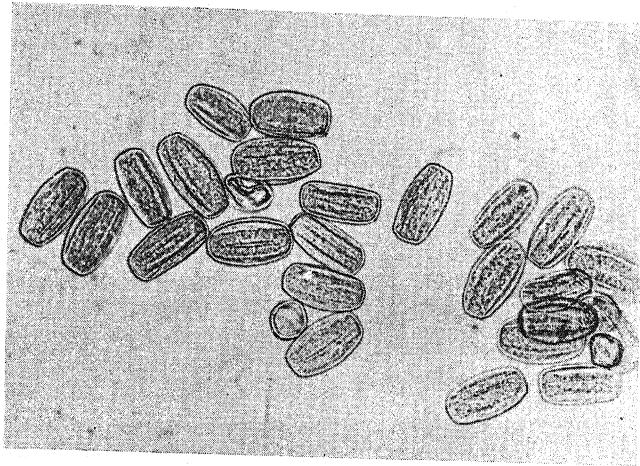


b

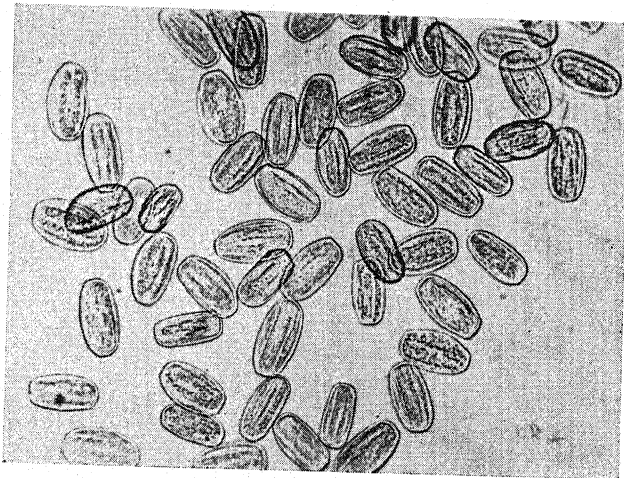
Fig. 1. Long pollen of mid-styled plant M_3 . *a*, central flower of dichasium, main inflorescence; wet soil. 4 August 1938. *b*, lateral flower of dichasium, lateral inflorescence; dry soil. 8 August 1938. Enlargement 300 \times .

are fertile with long pollen. The differences of style structure have not yet been studied.

Jost (1907) put forth the theory that the three types of styles of *L. Salicaria* might have different values of the suction tension of their



a



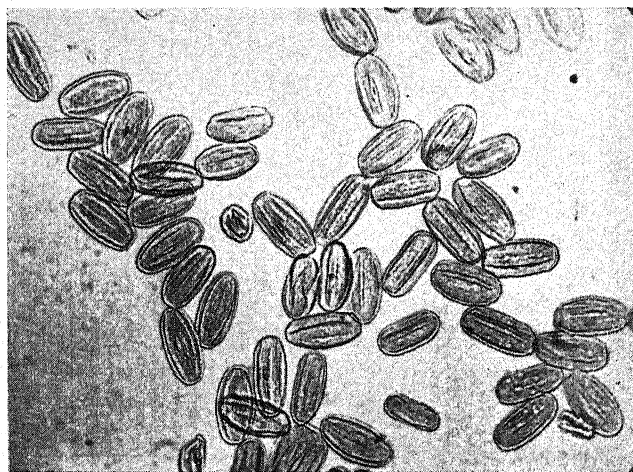
b

Fig. 2. Mid-pollen of short-styled plant S_1 . *a*, central flower of dichasium, main inflorescence, moist soil. 5 August 1938. *b*, lateral flower of dichasium, lateral inflorescence; moist soil. 5 August 1938. Note that the mid-pollen grains of the lateral flower are smaller or of the same size as the short pollen grains of the central flower of the mid-styled plant M_2 (Fig. 3*a*). They contain, however, more starch grains than the latter. Enlargement 300 \times .

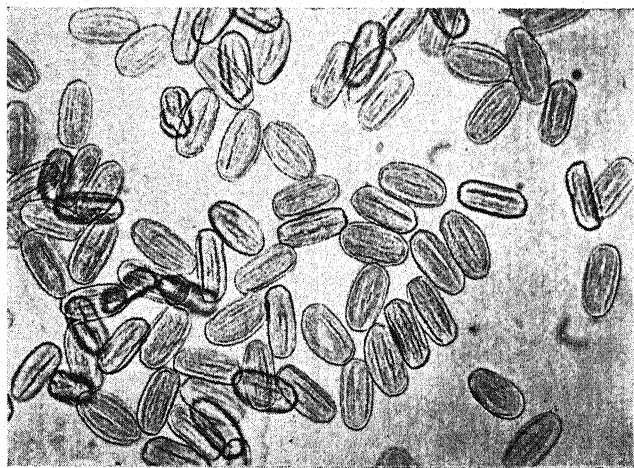
conductive tissue and correspondingly of the several pollen types. This, however, was not confirmed by my experiments (1937*a*, p. 67). Nor

400 *Influence of Nutrition upon Pollen Grain Size*

could a difference in the acidity of the stigmatic secretions be found in the three forms (1938*a*, p. 179). There are, of course, many more factors



a



b

Fig. 3. Short pollen of mid-styled plant *M*₂. *a*, central flower of dichasium, main inflorescence; wet soil. 4 August 1938. *b*, lateral flower of dichasium, lateral inflorescence; dry soil. 8 August 1938. Enlargement 300 ×.

that might influence the fertility of the different types of pollinations in *L. Salicaria*, and these factors are bound to be of a physiological nature. This can be deduced from the fact that from two to four pollen types are

found in the same flower (e.g. mid-styled flowers with long, short and one or two mid-stamens), each of which has its typical degree of fertility when placed on the different stigmas. The problem of fertility is here probably a purely physiological one. But it will be very difficult to find out what sort of substances are involved. New experiments on the fertility of *L. Salicaria* have been carried out in 1939 and will be continued, in order to trace the interactions of style structure and pollen contents.

SUMMARY

The three types of pollen grains in *Lythrum Salicaria* L. show a very wide range of variation and transgression. The pollen size of every type is influenced by nutritional conditions. The water supply of the plants makes itself felt in this respect, and especially the position of the flowers on the inflorescences. Differences of pollen diameters up to 6μ (average pollen length) were, for example, registered between central flowers of dichasiums of strong main shoots and lateral flowers of weak lateral shoots. Fertility, however, is dependent on certain quantitative differences of the pollen contents which do not seem to be in direct connexion with pollen size. The nature of these substances is as yet unknown.

This paper is published with the help of a subsidy from the Foundation Dr Joachim de Giacomini of the Swiss Society of Natural History. The writer is likewise indebted to Prof. M. Wildi, St Gall, for looking over this text.

REFERENCES

- BARLOW, N. (1923). "Inheritance of the three forms in trimorphic species." *J. Genet.* **13**, 133-46.
- BODMER, H. (1927). "Beiträge zum Heterostylieproblem bei *Lythrum Salicaria*." *Flora, Jena*, N.F. **22**, 306-41.
- DARWIN, CH. (1877). *The Different Forms of Flowers on Plants of the same Species*. London.
- EAST, E. M. (1927). "The inheritance of heterostyly in *Lythrum salicaria*." *Genetics*, **12**, 393-414.
- ERNST, A. (1936). "Heterostylie-Forschung. Versuche zur genetischen Analyse eines Organisations- und 'Anpassungs'-Merkmales." *Z. indukt. Abstamm.- u. Vererb.-Lehre*, **71**, 156-230.
- FERGUSON, M. C. & COOLIDGE, E. B. (1932). "A cytological and a genetical study of *Petunia*. IV. Pollen grains and the method of studying them." *J. Bot.* **19**, 644-58.
- JOST, L. (1907). "Ueber die Selbststerilität einiger Blüten." *Bot. Ztg.* **65**, 77-117.
- Journ. of Genetics* **XL**

402 *Influence of Nutrition upon Pollen Grain Size*

- KRUMBHOLZ, G. (1926). "Untersuchungen über die Scheckung der *Oenotheren*-bastarde, insbesondere über die Möglichkeit der Entstehung von Periklinalchimären." *Jenai. Z. Naturwiss.* **62**, 187-260.
- PIECH, K. (1922). "Ueber die Veränderlichkeit der Pollenkörner von *Linaria genistifolia* Mill. und einiger anderer Pflanzen." *Bull. Soc. Polon. Nat.* **47**, 412-82.
- SCHOCH-BODMER, H. (1937a). "*Lythrum Salicaria* L." *Lebensgesch. Blütenpfl. Mitteleur.*, Lief. 53/54, 9-76.
- (1937b). "Einfluss verschiedener Faktoren auf die Pollenbeschaffenheit bei *Lythrum Salicaria* L." *Verh. Schweiz. Naturforsch. Ges.* **118**, 149-50.
- (1938a). "Farbumschlag des Pollenanthocyans durch saure Narbensekrete bei *Lythrum Salicaria*." *Verh. Schweiz. Naturforsch. Ges.* **119**, 179.
- (1938b). "Die Veränderlichkeit der Pollengrösse bei *Lythrum Salicaria*." *Flora, Jena*, N.F. **33**, 69-110.
- STOUT, A. B. (1923). "Studies of *Lythrum Salicaria*. I. The efficiency of self-pollination." *Amer. J. Bot.* **10**, 440-9.
- TISCHLER, G. (1917). "Pollenbiologische Studien." *Z. Bot.* **9**, 417-88.
- (1918). "Untersuchungen über den anatomischen Bau der Staub- und Fruchtblätter bei *Lythrum Salicaria* L. mit Beziehung auf das 'Illegitimitätsproblem'." *Flora, Jena*, N.F. **11**, 162-93.
- UBISCH, G. v. (1925). "Genetisch-physiologische Analyse der Heterostylie." *Bibliogr. genet.* **2**, 287-342.

LINKAGE AND CROSSING-OVER IN THE HUMAN SEX CHROMOSOMES

BY THEODORE WHITE, PH.D.

Fellow of the University of Wales

(Statistical Appendix by Prof. J. B. S. HALDANE, F.R.S.)

(With Eleven Text-figures)

THE outstanding feature of modern research in human genetics is the emphasis laid by many workers on the necessity of obtaining data concerning the "linkage" of genes, evidence of which was first obtained for human beings by Madlener (1928). The results of the work by Morgan and his collaborators on *Drosophila melanogaster* are sufficient evidence of the importance of this phenomenon in relation to a proper understanding of the mechanism of heredity.

Haldane (1936), by statistical analysis of the data accumulated by previous workers regarding a group of hereditary defects, came to the conclusion that the data required the assumption that crossing-over of the X- and Y-chromosomes of man was an important factor in human heredity. Statistically, as was indicated by Fisher (1936), this conclusion can be regarded as a valid interpretation of the facts but, as Haldane (1936) himself indicated, no clear-cut case of chromosome crossing-over was then available in respect to human heredity. In the case of *Drosophila*, the study of abnormal ratios in the inheritance of linked defects has led to the construction of extremely detailed maps of the chromosomes of this organism and Haldane (1936), as a result of the work noted above, succeeded in mapping provisionally the loci of six genes on the pairing segment of the X-chromosome in man. He pointed out also that gene-linkage in man would undoubtedly be most easily recognized in the case of genes carried on the sex chromosomes and suggested, in view of the relatively high incidence of colour-blindness in the general population, that all persons known to any investigator to be suffering from sex-linked hereditary defects should be examined for colour-blindness. Such an example of colour-blindness in association with another sex-linked defect is available in the present work.

The study of the hereditary transmission of ocular defects in man has already led to the discovery of anomalies of which not the least important

404 *Linkage and Crossing-over in Human Sex Chromosomes*

is the fact that many of these defects are characterized by the existence of two distinct modes of genetic transmission. Punnett (1933) indicated that this situation has no close parallel in animal genetics, for the defects may be transmitted as simple Mendelian dominants or as sex-linked recessives, without any clinical differentiation between the types from these two sources. The existence of this anomaly is well instanced in the studies of night-blindness which are summarized up to 1932 by Bell (1932). The defects classed under this title fall into three major categories, the first including that form of night-blindness which results from vitamin A deficiency. It is capable of clinical treatment in that the defect disappears on restoring a normal vitamin balance in the diet, and is therefore genetically of little interest. The second category concerns the defect *Retinitis pigmentosa*, identified readily in that it is progressive, generally resulting in total blindness, and is characterized by distinct fundus changes which are readily observed by ophthalmoscopic examination of the affected person.

The third category, which is important in relation to the present work, concerns congenital stationary night-blindness. This form of the defect may be distinguished from that in the two preceding categories in that it is present from birth, is non-progressive, is not cleared up by vitamin A treatment, and in that ophthalmoscopic examination reveals no fundus changes that can be regarded as typical of the defect. This category is further subdivided by Bell (1932) according as to whether or not the defect is transmitted as

- (i) a Mendelian dominant, appearing in both males and females,
- (ii) a sex-linked recessive, appearing in the males only but transmitted by the females.

This latter subcategory is further divided according as to whether or not

- (a) the defect appears alone,
- (b) the defect is accompanied by myopia.

Myopia itself and colour-blindness, with which two defects the present work is also concerned, are again known to be transmitted in sex-linked fashion (cf. Bell, 1932), although this is not universally true of myopia.

Bell (1932, p. 195), in discussing these defects, stated: "When we look for other defects or anomalies characteristically associated with colour-blindness we can find no evidence at all that such exist... Associated eye defects appear to be extremely rare and of no apparent significance... our information is incomplete and may be misleading..."

nevertheless, other eye defects have been too frequently sought for to be overlooked if at all frequent and we are inclined to the view that colour-blindness is probably not characteristically associated with other hereditary defects. We might even tentatively suggest that perhaps these sex-linked hereditary defects, following their typical mode of descent, are as a group found to be characteristically associated with no other defects; such would appear to be the case for congenital stationary night-blindness (with myopia) and haemophilia."

Such a viewpoint, if correct, would have implied the necessity for formulation of a genetic hypothesis applicable only to human heredity, i.e. that the human *X*-chromosome can transmit only one gene for an abnormality. One would have had to assume that the other genes present were necessarily normal, or that the presence of more than one abnormal gene in the *X*-chromosome constituted a lethal factor, the association of two sex-linked defects never being observed owing to non-viability of the resultant organism. In view of the extensive advances, already made in genetical theory by that time, the publication of such a viewpoint in an authoritative work as late as 1932 on the basis of purely negative evidence merits some degree of criticism. It is perhaps poetic justice that the evidence now available for the linkage of such genes in man should be confined to just those three characteristics—night-blindness, colour-blindness, haemophilia—which, it was stated in 1932, never occurred in association.

Riddell (1937) instanced a pedigree in which haemophilia occurred in association with colour-blindness. Bell & Haldane (1937) instanced further cases in which the two defects were found in association. Only one case of crossing-over was noted, and that was not absolutely certain since the mother of the case in question may have been a mosaic due to somatic mutation. The linkage therefore was virtually, if not entirely, complete. Rath (1938) instanced a further pedigree in which a female known to be transmitting haemophilia acquired, apparently as the result of mutation, a gene for colour-blindness and gave rise to four sons, one normal, one colour-blind, one haemophilic, and one haemophilic and colour-blind, constituting definite evidence of the possibility of chromosome crossing-over in man. The data were not sufficient, however, to permit of an estimate of the frequency of crossing-over between the genes for these two defects.

In the present work, colour-blindness and congenital stationary night-blindness with myopia have been found in association in several members of a pedigree which extends over seven generations. The mode of occur-

rence of the defects necessitates acceptance of the fact that the genes responsible for these abnormalities must be located in the same X-chromosome and are transmitted as sex-linked recessives. It seems a logical conclusion that the early failure to observe such linkage must be ascribed to the comparative rarity of sex-linked defects, the still greater rarity of occurrence of associated sex-linked defects, and the difficulty of following up human pedigrees.

The present case is complicated by a high frequency of crossing-over of the maternal X-chromosomes, a feature which has resulted in the occurrence in the pedigree of night-blind, myopic, colour-blind males; colour-blind males; and night-blind, myopic males. It is clear from the subsequent evidence that, in regard to the possibility of linkage of defects and of chromosomal crossing-over, human heredity very definitely conforms to accepted principles of more general genetics—a fact which is of some scientific importance.

DISCUSSION OF THE PEDIGREE AND OF THE DEFECTS STUDIED

The pedigree covers seven generations and commences with a certain Mr Shurly (I. 2) who, it is almost certain, was night-blind, myopic, and perhaps colour-blind. This deduction, concerning an individual the date of whose birth was probably somewhere around 1760 to 1780, rests upon the fact that his granddaughter, Mrs Emily Clarkson (*née* Keates) (III. 11), informed her daughter Mrs B—1 (IV. 41) that Mr Shurly was blind. The rarity of spectacles until the early nineteenth century, combined with the incidence of night-blindness and a severe myopia of as much as -14 diopters in the present-day descendants of Mr Shurly, together with the fact that the said individual married and produced several children, renders it highly probable that he was not actually blind but that he did suffer from defects which, in the absence of spectacles and adequate night-time lighting, would have made his capacity for visual perception far less than normal. In the light of this, the information which the writer received from IV. 41 is significant and seems to point to Mr Shurly as the earliest known case of the defects in this family.

From the same source (IV. 41) came also the information that Mr Shurly had at least four children of whom one, a daughter, Eliza Mary Ann Shurly (II. 1) (7:3:1800-1886) married John (Jesse?) Keates (II. 2) and gave rise to the family under discussion. No trace of the remaining Shurly children has been found, but it is possible that they too may have given rise to descendants exhibiting linked ocular defects

in the same manner as the members of the present pedigree. The information regarding them is therefore given in view of the possibility that their descendants may be independently located and linked up to the present pedigree.

It seems certain from information given the author regarding John (Jesse ?) Keates by IV. 41 and by Mr H. G. B—1 (IV. 15) and Mrs C—e (IV. 16) who are the sole living descendants who remember having met him, that his vision was normal, or at least that he was not night-blind or myopic, a fact which lays the onus for transmission of these defects upon Eliza Shurly (II. 1) and links up with the statement regarding her father's "blindness". Little more is known of these members of generations I and II except that they lived in London, but in the case of generation III it has been possible to check the accuracy of some of the information concerning the members of this generation (all of whom are now dead) by reference to marriage and birth certificates which are available for III. 1 and III. 11 respectively.

Eliza Keates (*née* Shurly) (II. 1) had six children in the order Eliza Mary Ann Keates (III. 1) (18 : 12 : 1828—14 : 16 : 1919), Thomas Jesse Keates (III. 4), Alfred Keates (III. 7), Helen Keates (III. 8) (1832–1909), Rachel Keates (III. 10), and Emily Haines Keates (III. 11) (25 : 12 : 1843–1900). The significance of the pedigree emerges quite clearly on consideration of the descendants of Eliza Mary Ann Keates (III. 1) (Mrs Bull), the eldest of the children. Her husband, Seth Bull (III. 2), was not night-blind or myopic, a fact which has been confirmed by the testimony of several members of the family and from existing photographs, and it is quite certain that Eliza Keates was responsible for the transmission of deuteranopia and also night-blindness with a severe myopia.

The defects appear only in her male descendants, both separately and in association, and the form and extent of their occurrence makes it certain that not only are the defects sex-linked but that they were originally (and are still in some cases) located on the same X-chromosome. It is further obvious from the results that separation of the defects has occurred on several occasions, producing, in addition to the expected night-blind myopic colour-blind males, other males who are colour-blind only or night-blind myopic only—the occurrence of all three types being capable of explanation only if it is assumed that crossing-over of the XX sex-chromosomes can and does occur in the human female.

Throughout this branch of the family the type of colour-blindness found is deuteranopia or green blindness. Its presence in the affected

408 *Linkage and Crossing-over in Human Sex Chromosomes*

individuals has been shown by use of the Ishihara test and has been confirmed independently, on the author's behalf, in the case of several individuals by Mr Rudd, Senior Surgeon of the Birmingham and Midland Eye Hospital, using both Ishihara charts and the Edridge-Greene Lamp. Mr Rudd has also confirmed the myopia and the presence and type of night-blindness in the same individuals and thereby provided an invaluable independent testimony for which the writer is extremely grateful.

The writer has tested as many members of this branch of the family as possible. In the case of the majority of the affected individuals, independent confirmation of the findings and details of the eyesight of the members is available in the form of prescriptions obtained from the oculists who provided them with spectacles, or the specialists originally responsible for drawing up the prescriptions. Details are given in the observational section of this work.

As for the night-blindness found in this branch of the family, it is undoubtedly congenital stationary night-blindness. This follows from the sex-linked mode of its inheritance, the non-progressive nature of the defect (present from earliest recollection but of constant intensity throughout life), and from the complete absence of any signs of fundus degeneration or pigmentation (Mr Rudd's findings). These facts exclude *Retinitis pigmentosa*. The exclusion of any possibility that the defect results from vitamin A deficiency follows from the fact that one of the members, Mr W. W. W—n (V. 24), was given by the "Glaxo" Laboratories, 48,000 international units of vitamin A per day, in the form of "Prepalin" capsules, over a period of eighteen months without any appreciable improvement in his night-blindness. The dosage is some six times that generally accepted as required to clear up a vitamin A-deficiency night-blindness, and the period of treatment much longer than would be required. The author is grateful to the "Glaxo" Laboratories for permission to quote this datum as a confirmation of his findings. The negative result of this experiment may be emphasized here as a warning against the apparently growing tendency in medico-physiological circles to consider all forms of night-blindness as resulting from vitamin deficiency.

There is available, therefore, a variety of independent evidence confirming the writer's observation of the presence of deuteranopia and congenital stationary night-blindness with myopia in the descendants of Eliza Keates (III. 1). She gave rise to three sons (IV. 2. 8. 15) and nine daughters, and two at least of the daughters have abnormal X-

chromosomes produced as the result of crossing-over of the maternal XX-chromosomes—a fact which will be discussed later.

The eldest son, Charles Henry B—1 (IV. 2), was night-blind and severely myopic. His death occurred some years before the writer commenced this work, and it is impossible to say whether or not he was colour-blind. He left no descendants. The next son, Henry Seth B—1 (IV. 8), died in infancy, and there is no information concerning his eyesight. The youngest son, Henry George B—1 (IV. 15), was tested by the writer and found to have normal eyesight, and his descendants by his first wife are therefore of no interest to this discussion. His second marriage with his first cousin (IV. 41) who, as will be shown later, was transmitting deuteranopia, was consanguineous. She herself, like all the remaining female members of the pedigree, exhibited none of the defects studied. The description of the occurrence of the defect in the descendants of this marriage is, however, best deferred until the branch of the family of which IV. 41 is a member is considered.

Of the nine daughters of Eliza Keates, one (IV. 3) died in infancy, one (IV. 13) died unmarried, and one (IV. 11) although married has no progeny. They need not therefore be discussed further. The remaining six daughters (IV. 4, 6, 9, 16, 18, 20) are all transmitting the ocular defects, the high percentage of female transmitters in this generation being probably the result of chromosome crossing-over. Each has given rise to a group of descendants which must be discussed in some detail.

The eldest of these daughters, Emily Eliza (IV. 4) (Mrs P—s), had one son, Chris. P—s (V. 2), who is night-blind myopic and colour-blind.

The next daughter, Kate Ellen (IV. 6) (Mrs W—t), had three sons, one of whom died in infancy, while his twin is night-blind myopic and deuteranopic. The third son is not night-blind or myopic, but is in New Zealand and not available for a test of his colour perception.

The third of the six daughters, Alice Clara (IV. 9) (Mrs W—), has given rise to the most important branch of the family. Of her five sons, Clifford Augustus (V. 11) is night-blind myopic and colour-blind as are also his brothers, John Wilfred (V. 15) (the propositus) and William (V. 24). Walter (V. 19) is colour-blind only, while Leslie (V. 22) is night-blind myopic but not deuteranopic. Since the father of these sons (IV. 10) has been tested and found not to be colour-blind he does not enter into the interpretation. It is obvious, considering the mode of occurrence of the three defects in these five sons, in conjunction with its occurrence in other branches of the family, that Alice Clara B—1 (IV. 9) must have carried on one of her sex-chromosomes determinant genes for all three

410 *Linkage and Crossing-over in Human Sex Chromosomes*

defects. This chromosome has been passed on intact to three of her sons. In the case of the other two sons, chromosome crossing-over has obviously come into play, causing one of them to be deuteranopic only, and the other to be night-blind myopic but not deuteranopic.

Further, two at least of the five sisters of these brothers are also transmitting the same defects. The eldest sister, Nell (V. 12) (Mrs P—k), has two sons, one of whom, Karl P—k (VI. 13), is night-blind myopic but not colour-blind. His brother, Sydney (VI. 16), has normal eyesight. These members, resident at Toronto, were tested for the writer by Dr J. W. MacArthur of the Toronto University Biology Department, and the writer is extremely grateful for his assistance in this matter.

Three of the remaining four sisters have not produced male issue, but the fourth, Dorothy W—n (V. 25) (Mrs G—y), has one son, Trevor (VI. 27), who is deuteranopic, but not night-blind or myopic, implying a further case of chromosome crossing-over in his or his mother's development. His brother died in infancy, and it is impossible to say whether or not he shared the defect. This section of the family therefore exhibits four indisputable cases of chromosome crossing-over and three cases of normal transmission.

To return to the three remaining daughters of (III. 1). The next, Florence Amelia B—l (IV. 16) (Mrs C—e), has produced six children including two sons, Harry John (V. 44) and Edgar (V. 46), both of whom are deuteranopic only, a result that can again be due only to chromosome crossing-over, either in their formation or in that of their mother. Their four sisters have given rise to only one son, Douglas S—n (VI. 43), who has normal eyesight.

The next sister, Maud Eugenie B—l (IV. 18) (Mrs J—s), has produced eight sons and six daughters, but it is impossible to say whether any of the latter are transmitters since they are all devoid of male issue. The first of the eight sons, Samuel (V. 55), died in infancy, and the condition of his eyesight is unknown. Three of the sons, Jack (V. 67), Chris (V. 70) and Roy (V. 73), were found to possess normal eyesight, whereas the remaining four, Albert (V. 57), Laurence (V. 64), Harry (V. 68) and Leslie (V. 75), were all night-blind myopic. No trace of deuteranopia was found in this branch of the family, and it seems almost certain that chromosome crossing-over took place in the development of the mother (IV. 18), making it possible for her to transmit only night-blindness and myopia.

Two further cases of crossing-over are found again in the last section of this branch of the family which requires discussion, the descendants of

Emmeline Maria B—l (IV. 20) (Mrs W—e and later Mrs W—n). Of her three sons, the eldest, Albert W—e (V. 79), is night-blind myopic, but not deuteranopic. The next son, Jack (V. 81), lives in America and has not been tested for deuteranopia but is known to be free from night-blindness and myopia. The third son, Leonard (V. 82), is stated to have had normal eyesight but died of tuberculosis some years ago, and had not been tested for deuteranopia. Of their four sisters, Maud (V. 76) (Mrs V—y) has two sons with normal eyesight. The other married sister, Helen (V. 83) (Mrs M—e), has one son, Brian (VI. 61), who was found to be deuteranopic only, a further case of chromosome crossing-over.

In this branch of the family (the descendants of Eliza Keates (III. 1)) occurs also the only case of colour-blindness which has been introduced into the family from an extra-familial source—that of David W—n (VI. 22), who suffers from incomplete red-green blindness—a form of colour-blindness easily distinguished in the Ishihara test from the green blindness characteristic of other members of the pedigree. Since his father (V. 22) happens to be the one of the five brothers discussed earlier who is night-blind myopic but *not* colour-blind, VI. 22's colour-blindness obviously derives from his mother (V. 21) who is not in the direct line of the family descent.

This poses an interesting question in that VI. 22 has a sister, Monica W—n (VI. 23), who will undoubtedly have received from her father (V. 22) one *X*-chromosome bearing the genes for night-blindness and myopia. She has a 1 in 2 chance of receiving from her mother an *X*-chromosome bearing a gene for colour-blindness. It is evident from the data given above that crossing-over of the *X*-chromosomes between the loci of these two genes is very frequent, and it is possible, therefore, that if VI. 23 eventually gives rise to male children, some of them may be found to be night-blind myopic *and* colour-blind despite the fact that owing to chromosome crossing-over her father's descendants should be free from colour-blindness.

A similar possibility is present in another section of this branch of the family—peculiarly enough, again where crossing-over has caused colour-blindness to be no longer a feature of the inheritance—i.e. in the children of IV. 18. The two brothers, Roy J—s (V. 73) and Leslie (V. 75), have married two sisters (V. 72 and 74) whose father is known to be colour-blind. The potential daughters of V. 75 and V. 74 will receive *X*-chromosomes with genes for night-blindness and myopia from their father (V. 75) and have a 1 in 2 chance of receiving an *X*-chromosome with a gene for colour-blindness from their mother (V. 74). The grandsons of V. 75 may

412 *Linkage and Crossing-over in Human Sex Chromosomes*

therefore again exhibit night-blindness and myopia in association with colour-blindness. It is a curious reflexion upon the laws of probability that such a situation (i.e. the reintroduction of colour-blindness into the family) should occur in each case in sections of the family from whose inheritance the deuteranopia has been eliminated. This feature, with the fact of the presence of several young unmarried or newly married daughters of night-blind myopic deuteranopic males in the present living generation, should make the history of the next few generations of this family well worth study. For this reason, fuller details of the pedigree than can be reproduced here are being deposited with the Bureau of Human Heredity, 115 Gower Street, London, with a view to the family being available for further study by any future investigators who may care to undertake the task. Pedigrees such as the present one will undoubtedly be rare and the details available will permit of a full follow-up of the present investigation.

- It is necessary now to return to consideration of the sections of the pedigree containing the descendants of the remaining children of Eliza Shurly (II. 1). Of these children, Thomas Jesse Keates (III. 4) is stated by Mr S—t (IV. 23), his daughter's husband, by Mr W. C. W—n (IV. 10), by Mrs W—s (IV. 14) and by Mrs C—e (IV. 16), all of whom knew him well, to have had normal eyesight, with no myopia or night-blindness, and never to have used spectacles. He may of course have been colour-blind, as a result of the chromosome crossing-over which has already been shown to be of frequent occurrence, but there is no evidence on the point. Unfortunately, he has no living descendant in whom colour-blindness could be present as a result of hereditary transmission from him, and the group of his descendants shown in the pedigree chart need not therefore be discussed here.

Alfred Keates (III. 7) is also known not to have suffered from night-blindness or myopia, and again there is no knowledge as to whether or not he was colour-blind. He is reported by IV. 16 and IV. 41 to have married, but it is uncertain whether or not he gave rise to descendants, and for some reason none of the family have been able to provide any further information which would enable a decision as to the point.

Rachel Keates (III. 10) died unmarried at the age of fourteen and therefore needs no discussion. Of the two remaining sisters, Helen Keates (III. 8) became Mrs Long, and gave rise to one daughter (IV. 28), who cannot be traced, and four sons (IV. 31. 33. 36. 39), none of whom were myopic or night-blind. Information is available regarding the numbers of their descendants, but such of them as can be traced are free

from ocular defects and it is probable that Helen Keates (III. 8) was not transmitting the defects characterizing the family. There is no evidence that she transmitted colour-blindness, but it has not been possible to locate and test sufficient of her descendants to exclude definitely such a possibility. Again, further discussion of this section of the pedigree would serve no useful purpose.

In the case of the remaining sister, Emily Keates (III. 11) (Mrs Clarkson), the circumstances are different. Of her four sons (IV. 40. 42. 44. 48), two (IV. 40. 48) died in infancy. Charles Alfred (IV. 44) was tested by the writer and was found to have normal eyesight. His brother, Samuel Henry Thomas (IV. 42), died of enteric fever while fighting in the Boer War and is stated by IV. 41 and IV. 44 to have had normal eyesight, although it is again impossible to state definitely that he was not colour-blind.

Two of Emily Keates's daughters have married, one, Emily Ann Eliza (IV. 41), having married her first cousin, Mr H. G. B—l (IV. 15), and given rise to two daughters and two sons (V. 37. 40. 41. 42), the younger of the latter being green-blind, but not night-blind or myopic. The defect may have been inherited from his grandfather, Mr Clarkson (III. 12), of whom, being dead, it is impossible to say he was not deuteranopic, but—in view of the frequency of occurrence of deuteranopia in the descendants of his grandmother's sister it seems fairly certain that Emily Keates (III. 11) was transmitting deuteranopia received via Eliza Shurly from Mr Shurly. If this conclusion be correct, it implies that in formation of the ovum which gave rise to Emily Keates (III. 11) or her daughter Emily C—n (IV. 41), crossing-over of the XX-chromosomes occurred, allowing of the transmission of deuteranopia unaccompanied by night-blindness and myopia. This conclusion seems hardly disputable in the light of the evidence already given earlier concerning the descendants of Eliza Keates (III. 1).

The remaining daughter of Emily Keates, i.e. Kate Clara Alice (IV. 46), has produced one son and a daughter. Her son is not night-blind or myopic but may be colour-blind. This, however, cannot be determined, since he is at present in Vancouver and is not available for test.

Since Prof. J. B. S. Haldane, F.R.S., has very kindly consented to provide a statistical appendix to this work giving a fuller mathematical treatment of the linkage and cross-over ratios than the writer is capable of providing, it is necessary here to add only a few remarks concerning this aspect of the pedigree.

414 *Linkage and Crossing-over in Human Sex Chromosomes*

The pedigree contains 273 individuals of whom 183 are in the direct line of descent from Mr Shurly (I. 2). Of these latter, forty-five are deceased, and twenty-eight owing to emigration were not available for test. Of the remaining 110 members in the direct line of descent, seventy-one (i.e. 64.6 %) have been interviewed and tested by the writer or by other competent persons on his behalf, the majority of the untested members available in this country being of little, if any, interest to the study (e.g. females with no male issue). In all, ninety-nine members of the pedigree have been tested, most of the twenty-eight extra-familial members having been tested to ensure that they were not responsible for introducing any of the defects from an extra-familial source.

Of the 183 direct members of the family, eighty-seven are males and ninety-six females. Thirteen of these latter have produced sons or grandsons exhibiting the defects and are thus known to be transmitters. A further nine—daughters of males with the defects—will, in the normal course of events, be found to be transmitters when they marry and produce progeny. The ninety-six females therefore include at least twenty-two transmitters (i.e. 22.9 %), while of the eighty-seven males, and their forerunner (I. 2), twenty exhibit the ocular defects (i.e. 22.7 %), a surprisingly close correspondence. Of these twenty males, five are night-blind, myopic, deuteranopic; seven are night-blind, myopic but not deuteranopic; six are deuteranopic only; a further two (both dead) are known to be night-blind myopic, while data concerning their perception of colour is not available.

The thirteen females producing sons or grandsons with the defects have produced thirty-eight sons, of whom nineteen (Mr Shurly I. 2 is excluded here) are so affected, but thirteen of the thirty-eight sons, owing to death or emigration, cannot be said to be free from deuteranopia and have to be disregarded. The defects therefore are exhibited by 76 % of the sons for whom data is available, the 26 % excess over the expected 50 % being due probably to the lack of complete data, and to the high degree of chromosome crossing-over.

Of the forty-two daughters of the thirteen transmitting females, twelve have produced progeny with the defects, but only three can be said with some certainty to be non-transmitters, seven having not married, nine having died unmarried, while twenty-three have no male issue, or have produced too few sons to permit of a decision as to whether or not they are transmitting.

If II. 1 be assumed to be transmitting the genes for both colour blindness (*c*) and night-blindness myopia (*n*) on the same X-chromosome,

then the cross-over ratio between these two genes can be determined empirically within certain limits by consideration of Tables I and II. This assumption, as can be seen from consideration of Prof. Haldane's statistical appendix, is not necessarily correct, since II. 1 may have the genetic constitution $\frac{++}{cn}$ (if both defects are derived from her father),

$\frac{c+}{+n}$ (if the colour-blindness comes from her mother and night-blindness from the father), or $\frac{++}{++}$ (if the colour-blindness is introduced by her

TABLE I

*Giving the lowest possible value for the cross-over ratio
between the genes c and n*

	Daughters		Sons	
	Transmitting night-blindness, myopia, deuteranopia (i.e. non- cross-overs)	Transmitting night-blindness and myopia, or only deuteranopia (i.e. cross-overs)	Night-blind, myopic, and deuteranopic (i.e. non- cross-overs)	Night-blind, myopic, or only deuteranopic (i.e. cross-overs)
Mother				
II. 1	III. 1 III. 11			
III. 1	IV. 4 IV. 6 IV. 9 IV. 20	IV. 16 IV. 18		
III. 11	IV. 41			
IV. 4			V. 2	
IV. 6			V. 4	
IV. 9	V. 27	V. 12	V. 11 V. 15 V. 24	V. 19 V. 22
IV. 20	V. 83			V. 79
IV. 41				V. 42
V. 27				VI. 27
V. 83				VI. 61

husband (II. 2)). It seems most probable, however, that II. 1 had the genetic constitution $\frac{++}{cn}$, and although the cross-over ratio, assuming this condition, is found to be lower than that obtained later by Prof. Haldane's more detailed analysis, it is still of the order of 43%, a significantly high value.

In Table I III. 11, V. 25, and V. 83 are presumed to be capable of transmitting all the defects. In consequence, ten females have produced nine females and five males normally, and three females and six males

416 Linkage and Crossing-over in Human Sex Chromosomes

by processes involving cross-over of the XX-chromosomes. The cross-over ratio is therefore 39.1 % (nine of twenty-three children).

Table II permits deduction of the upper limit of the cross-over ratio by assuming crossing-over to have occurred in the development of III. 11, V. 25 and V. 83 instead of in that of their sons or of their daughters or granddaughters who are transmitting the defects.

TABLE II
Giving the highest possible value for the cross-over ratio
between the genes *c* and *n*

	Daughters		Sons	
	Transmitting night-blindness, myopia, deuteranopia (i.e. non- cross-overs)	Transmitting night-blindness and myopia, or only deuteranopia (i.e. cross-overs)	Night-blind, myopic, and deuteranopic (i.e. non- cross-overs)	Night-blind, myopic, or only deuteranopic (i.e. cross-overs)
Mother				
II. 1	III. 1	III. 11		
III. 1	IV. 4 IV. 6 IV. 9 IV. 20	IV. 16 IV. 18		
IV. 4			V. 2	
IV. 6			V. 4	
IV. 9		V. 12 V. 27	V. 11 V. 15 V. 24	V. 19 V. 22 V. 79
IV. 20		V. 83		

In this case six females have produced five females and five males normally, and six females and three males by processes involving chromosome cross-over. The cross-over ratio is therefore 47.4 % (nine of nineteen children).

This admittedly empirical treatment indicates the cross-over ratio to be within the extreme limits 39.1-47.4 %, or, taking the mean of these values, to be 43.25 ± 4.15 %. If this value which, for reasons given above, is lower than that (64.8 %) given by proper statistical treatment be taken as correct, 71.7 % of the sons of the transmitting females should exhibit the defects instead of the expected 50 %, a fair agreement with the observed 76 % given earlier. Further, it follows that the human X-chromosome map must be a long one, as in *Drosophila* spp. and *Gallus*, and not short as in *Lebistes*. Taking the length of the human X-chromosome as 100 units, the present work therefore indicates the distance between the gene for deuteranopia, and that (or those) for night-blindness and myopia, to be some 43.25 ± 4.15 units. Since, in no case, have any of these defects been transmitted by an affected male to his sons, it

can be said also with some certainty that the genes for deuteranopia and night-blindness with myopia are located on the non-pairing segment of the X-chromosome, i.e. the section which does not cross-over with the male Y-chromosome (a behaviour which Haldane (1936) states is possible). It cannot be said whether or not the night-blindness and its accompanying myopia are both governed by the same gene, for in no case has the one defect been found without the other. Myopia does occur in certain males in the family without an accompanying night-blindness or deuteranopia, but in all such cases the degree of the myopia is very small (generally *ca.* -1 diopter), and in most its occurrence can be traced to an extra-familial source. There is no evidence of any crossing-over between the genes for night-blindness and myopia, nor is there any evidence in any other pedigree known to the writer, and it can be stated with certainty that these two defects are either governed by the same gene, or by two genes in positions so closely adjacent that the cross-over ratio between them is very small. In consequence of this it has been possible to simplify discussion of the crossing-over in this work by assuming only two genes to be concerned—one determining deuteranopia and 43.25 ± 4.15 units distant from that determining the occurrence of night-blindness with myopia.

As for the defects themselves, it will be seen from consideration of the details in the observational section that their intensity varies to some extent. No quantitative estimation of the degree of night-blindness in any of the individuals has been possible owing to the non-availability of apparatus suitable for such estimation. It is believed, however, that the capacity for receiving weak light impulses is a function of the peripheral rods of the retina, and in this respect certain findings made by Mr Rudd are important. In his tests on V. 4. 11. 15. 22, Mr Rudd noted, in three of the four cases, a general contraction of the fields of vision, which contraction can be observed in the Perimeter and Scotometer Charts illustrated in the observational section of this work. This contraction was scarcely noticeable in the case of V. 4, whose night-blindness, however, is not as severe as in the other three cases. A similar contraction was observed by Dr J. X. Robert of Toronto in the case of VI. 13.

In the case of all the night-blind individuals seen by the writer, it was stated that they had suffered from the defect from their earliest recollection, and that its intensity had always seemed approximately the same. V. 11 and V. 24, however, both stated that the night-blindness appeared less troublesome when they felt in a really good state of health,

418 *Linkage and Crossing-over in Human Sex Chromosomes*

but other members failed to confirm this impression. Several of the affected individuals stated also that they "could not have too much light", and V. 11 noted, while on a Mediterranean cruise, that on occasions when other passengers had to use dark spectacles because of the intensity of the sunlight, he himself had never before experienced such a high degree of visual acuity. It seems clear, therefore, that even in the presence of what would constitute an excessive source of light to a normal individual, the night-blind person still cannot perceive the light intensity at its full value. This feature does not appear to have been noted by previous observers and is significant in its implication that the perception of light intensity by a night-blind person does not at any time attain the capacity for perception of light intensity of a normal-sighted individual—this applying not only when the light source is weak but also when it is excessive. If anything, these observations agree with the theory that light perception is a function of the peripheral rods and the visual purple pigment, and that hereditary night-blindness is due to a congenital deficiency in the amount of visual purple pigment available in the retina. It has already been pointed out that this hereditary deficiency *cannot* be compensated by vitamin A treatment, and it is possible that the pigment deficiency is physiologically due to a congenital incapacity to utilize vitamin A in the production of visual purple.

The degree of myopia present in the affected individuals is also subject to some fluctuation, varying from -2 diopters in V. 4, -6 diopters in V. 24, -7 in V. 75, to -9.5 — -14 in V. 15. 22. 57. 68. There is some evidence, too, that the degree of the defect becomes greater as the affected individual grows older—contrary to the general principle that the focal length of the eye increased with age—for V. 57 exhibited a myopia of -8 diopters in 1898, and -9.5 in 1930; V. 15 had a myopia -8.5 in 1931, and -10.5 in 1937; while V. 68 had a myopia of -12 in 1924, and -14 in 1933. Such comparisons, unfortunately, are not available for more of the affected individuals.

The degree of deuteranopia shows somewhat less variation, the majority of the affected males being quite distinctly green-blind. In a few cases, however, the individuals on being tested with the Ishihara charts were able on tests 22–25 to read the numbers which the green-blind individuals cannot generally distinguish, although in all such cases the individuals took some time to read the numbers distinctly, and stated that they were much less distinct than the accompanying figures, which are easily visible to the deuteranopic. V. 2, however, proved somewhat abnormal in being apparently totally colour-blind, although

this may perhaps be due to the effects of an accompanying nystagmus. There is distinct evidence therefore that the actual degree of intensity of the defects varies considerably in individuals who are undoubtedly deriving their genes for these defects from the same ultimate source. Whether or not this variation is due to slight alterations in the gene structures during their transmission it is impossible to say. It is clear, however, that such variation, together with the elimination of one or other of the defects in some of the members as a result of chromosome crossing-over, shows that evolutionary processes are by no means at a standstill in the human species. Under more unfavourable environmental conditions such changes might well affect the survival value of an individual.

In most but not all of the cases the night-blindness and myopia were found to be accompanied by marked astigmatism, and one individual also exhibited cataract, and two others nystagmus. The normal members of the family were surprisingly free from such alternative eye defects, except in one or two small groups.

OBSERVATIONAL

The following relevant data were acquired concerning the affected individuals, their colour perception being determined by means of the Ishihara charts involving the reading of twenty-five test numbers. Normal-sighted individuals give readings different from those given by deuteranopes, the two sets of readings being as follows:

Normal	Deuteranopic	Normal	Deuteranopic
12	12	5	—
8	3	7	—
6	5	16	—
29	70	73	—
57	35	—	5
5	2	—	2
3	5	—	45
15	17	—	73
74	21	26	2
2	—	42	4
6	—	35	3
97	—	96	9
45	—		

In the observational details, readings signifying deuteranopia are underlined:

I. 2. Mr Shurly, late eighteenth century. Stated by his great-granddaughter (IV. 41), on information from her mother (III. 11), to have "been blind"—probably night-blind myopic.

IV. 2. Charles Henry Bull (5. 12. 1852–1916). Hairdresser, Birming-

420 *Linkage and Crossing-over in Human Sex Chromosomes*

ham. Stated by his sisters (IV. 16 and IV. 18), his brother (IV. 15), his nephew (V. 15), his brother-in-law (IV. 10), to have been night-blind and severely myopic. The latter recollects "piloting him by the arm through the streets after dark". There is no data concerning his colour perception.

V. 2. Chris P—s. University lecturer, Brussels. Tested January 1940 for the author by P. I. Wilson, B.Sc. Night-blind, myopic and totally colour-blind, the Ishihara charts appearing to him as a "blur" in which the coloured numbers could not be differentiated. M. Copper (optician) of Brussels gave the following data regarding his vision when tested in 1935: R. -7 sph., -0.75 cyl., 75° ax.; L. 7 sph., -0.75 cyl., 75° ax.

V. 4. Norman W—t (1883—). Architect's designer, Birmingham. Night-blind, myopic and incomplete deuteranopic. His readings in the Ishihara test were: 12, 8, 6, 79, 37, —, 17, 71, —, 5, 87, —, —, 7, —, —, —, —, —, 2, 4, (2), 3 (5), 3, (6). Tested in July 1939. Aet. 56. The brackets in tests 23–25 indicate the reading of the enclosed figure to have been delayed and the underlined tests are those indicating red-green or green-blindness. Mr Harrison (optician) of Birmingham gave the following data regarding his eyesight when tested in 1937: R. -2 sph., -3.75 cyl., 120° ax.; L. -1.75 sph., -3.75 cyl., 40° ax.; Va = 6/18. He was examined for the writer by Mr Rudd, Senior Surgeon of the Birmingham and Midland Eye Hospital, on 26 July 1939, who stated: "History of Night-blindness—vision with correcting lenses R. 6/12, L. 6/9; incomplete red-green blindness—Fundi show no evidence of *Retinitis pigmentosa*—Fields of vision full."

Figs. 1 and 2 are the perimeter and scotometer chart readings for the right and left eyes respectively of V. 4 as provided by Mr Rudd.

V. 11. Clifford Augustus W—n (1881—). Pianist, Birmingham. Night-blind, myopic and deuteranopic—the myopia *ca.* -13. He was tested with Ishihara charts on 7 July and again in August 1939, giving the same readings each time: 12, 3, 6, 70, —, 2, 5, 17, 71, (2), (6), —, —, —, —, —, —, 2, 4, 3, 9. Mr Wood-White (Late Senior Surgeon of the Birmingham and Midland Eye Hospital) tested him on 24 April 1917 stating "very high myopia with consequent nystagmus and night-blindness. R. 6/24 (corr.), L. less than 6/60 (corr.)." Mr Rudd tested him on 2 August 1939 and stated "History of difficulty in getting about in dim light—vision with correcting lenses R. 4/12, L. 6/60—Fields of vision: moderate general contraction—Fundi: extensive myopic degeneration—Nystagmus—appears to be an incomplete red-green blind."

Figs. 3 and 4 give the perimeter and scotometer chart readings provided by Mr Rudd.

V. 15. John Wilfred W—n. Architect, Birmingham. Night-blind, myopic, deuteranopic. Tested June 1939 and gave the Ishihara readings: 12, 3, 5, 70, 35, 2, 5, 17, 21, =, =, =, =, =, =, =, =, 5, 2, 45, 73, 2, 4, 3, 9. Mr Harrison (optician), Birmingham, tested him in 1931 and found R. -8.5 sph., -1.75 cyl.; Va 8/18; L. -8.5 sph., -2.5 cyl.; Va 8/18. He was tested again on 12 September 1937 giving R. -10.5 sph., -2.0 cyl., 90° ax.; Va 8/24; L. -8.0 sph., -3.0 cyl., 90° ax.; Va 8/24. Mr Rudd tested him on 26 July 1939 and stated: "History of night-blindness—Vision with correcting lenses R. 6/18, L. 6/18—Fields of vision: some general contraction—Fundi: myopic crescents—incomplete red-green blind." Figs. 5 and 6 are the relevant perimeter and scotometer charts.

V. 19. Walter W—n (20. 11. 1890—). Manufacturer, Birmingham. Deuteranopic only. Tested in July 1939 giving the Ishihara readings: 12, 3, 5, 70, 35, 2, 3, 17, 21, =, =, =, =, =, =, =, =, 5, 2, 45, 73, 2, 4 (2), 3 (5), 9. He showed no evidence of night-blindness or myopia.

V. 22. Leslie W—n (1897—). Manufacturer, Birmingham. Night-blind, myopic but not colour-blind. He was tested in July 1939 and gave quite normal Ishihara readings. Mr Harrison (optician), Birmingham, examined him on 3 June 1937 and found R. -12 sph., -0.75 cyl., 60° ax.; L. -11, and stated cataract and iritis to be present also. Mr Rudd examined him on 2 August 1939 and stated: "History of defective vision especially in dim light—Vision with correcting lenses R. 6/18, L. 6/18—Fundi: some myopic degeneration round the disc—Fields of vision: slight nasal contraction only: Colour vision normal." Figs. 7 and 8 are the relevant perimeter and scotometer charts.

V. 24. William W—n (13. 6. 1899). Advertising agent, London. Night-blind, myopic and deuteranopic. Tested on 5 November 1939 he gave the Ishihara readings: 12, 3, 5, 70, 35, 2, 5, 17, 21, =, =, =, =, =, =, =, =, 5, 2, 45, 73, 2, 4, 3, 9. His optician's report gave R. -6.5 sph., L. -6.75 sph., -0.75 cyl., 80° ax.: the date of the test being 26 January 1939. He was given 48,000 units of vitamin A per day for 18 months by the "Glaxo" Laboratories without any noticeable improvement in his night-blindness.

V. 42. Frank Edward B—l (1917). Army, Torquay. Deuteranopic according to his parents and several of his cousins. Owing to the war he was not available for testing. No night-blindness or myopia.

422 *Linkage and Crossing-over in Human Sex Chromosomes*

— Normal field of vision. - - - - - Observed field. R. Right eye. L. Left eye.

26. 7. 39. $\frac{1}{2}^{\circ}$ white obj.

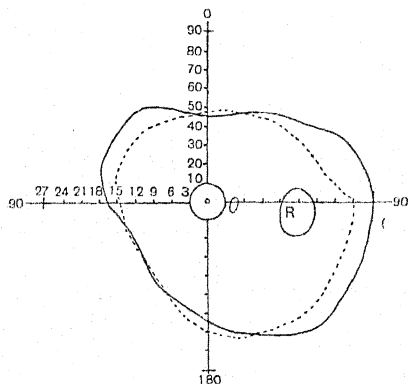


Fig. 1.

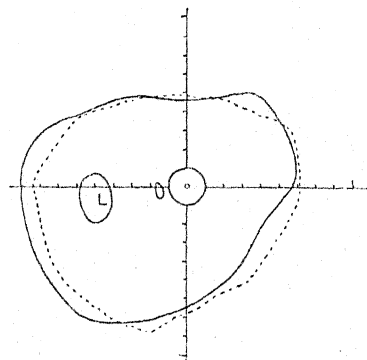


Fig. 2.

2. 8. 39. 2° white obj.

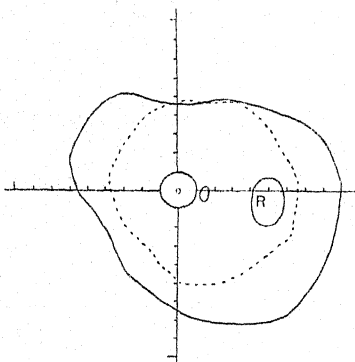


Fig. 3.

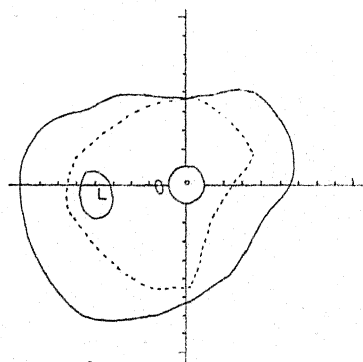


Fig. 4.

26. 7. 39. $\frac{1}{2}^{\circ}$ white obj.

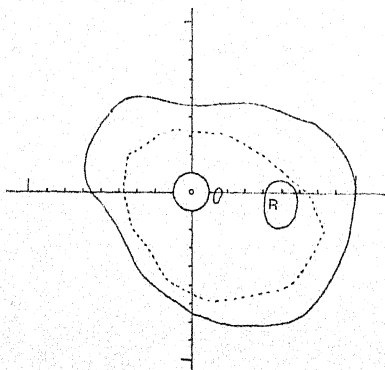


Fig. 5.

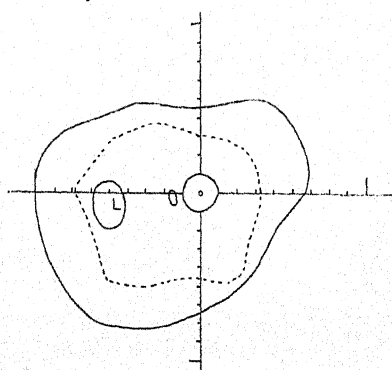


Fig. 6.

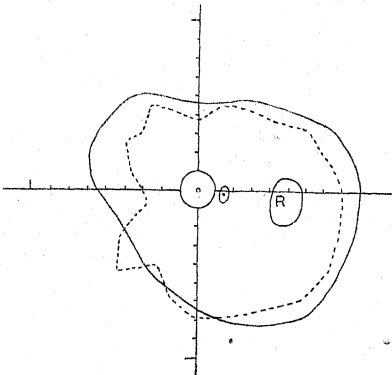
2. 8. 39. $\frac{1}{2}^\circ$ white obj.

Fig. 7.

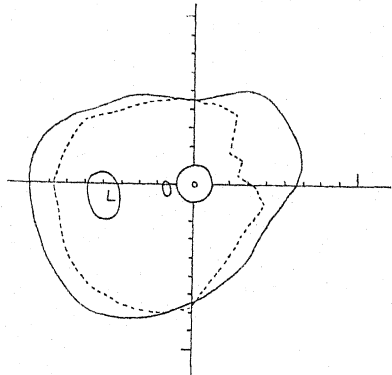


Fig. 8.

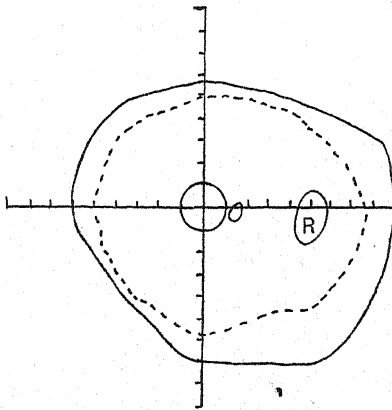
15. 1. 40. 1° white obj.

Fig. 9.

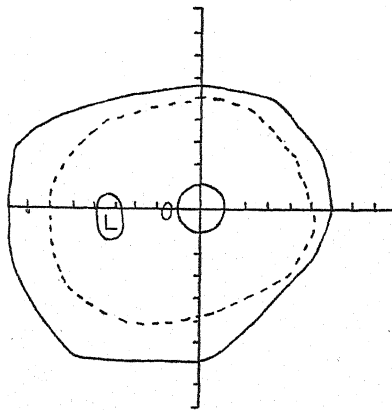


Fig. 10.

V. 44. Harry John C—e. Air Force, Birmingham. Deuteranopic according to the R.A.F. report, but passed for the ground staff. Owing to the war he again was not available for testing, but his wife states he cannot distinguish green from blue, or purple from red, and the author, visiting him at night when the Ishihara charts could not be utilized, verified this statement. No night-blindness or myopia.

V. 46. Edgar C—e. Mechanic, Coventry. Deuteranopic. Tested on 6 January 1940, he gave the Ishihara readings: 12, 3, 5, 70, 35, 2, 5, 17, 21, =, =, =, =, =, =, =, =, 5, 2, 45, 73, 2, 4, 3, 9. No night-blindness or myopia.

V. 57. Albert Alexander J—s (16. 9. 1894—). Carpenter, Leamington Spa. Night-blind, myopic, but not deuteranopic. Tested on

424 *Linkage and Crossing-over in Human Sex Chromosomes*

7 January 1940, he gave normal Ishihara readings. Mr Lloyd-Owen of the Birmingham and Midland Eye Hospital tested him on 29 April 1898, giving R. -8 sph., L. -8 sph. Mr Charnley and Sons (opticians), Leamington Spa, tested him on 2 January 1930, giving R. -9.5 sph., L. -9.5 sph.

V. 64. Lawrence J—s (10. 7. 1899-). Gardener, Par. Night-blind, myopic, but not colour-blind. Tested on 1 December 1939, he gave normal Ishihara readings. Mr Huddy (optician), St Austell, gave the following data for his eyesight: R. -3.5 sph., -1.5 cyl., 100° ax.; L. -5.5 sph., -1.5 cyl., 80° ax.

V. 68. Harry J—s (24. 11. 1903-). Gardener, Par. Night-blind, myopic, but not colour-blind. Tested on 1 December 1939, he gave normal Ishihara readings. Mr East, of the Royal Cornwall Infirmary, tested him on 23 August 1924 and found R. -12 sph., L. -11 sph. Mr Rolston (optician), Plymouth, tested him on 6 October 1933 and found R. -14 sph., L. -14 sph.

V. 75. Leslie J—s (29. 2. 1912-). Gardener, St Austell. Night-blind, myopic, but not colour-blind. Tested on 2 December 1939, he gave normal Ishihara readings. Mr Hodge (optician), Truro, tested him on 23 November 1933 and found R. -7 sph., -3 cyl.; L. -7 sph., -3 cyl.

V. 79. Albert W—e. Administration, Singapore. Information regarding V. 79 has been provided by Dr A. D. Williamson, F.R.C.S., Ophthalmic Surgeon and Physician, General Hospital, Singapore, who tested him for the author on 26 January 1940, and states: "I have examined Mr A. E. W—e to-day. Eyes are normal externally; pupils equal and react normally; ocular movements full. Visual acuity: R. -11.5 sph. -2.0 cyl. 123° ax. = 6/12 partly. L. -12.0 sph. -2.0 cyl. 65° ax. = 6/12 partly. Fundi show no abnormality beyond slight myopic crescents. With the ophthalmoscope there is detected a slight constant horizontal oscillatory nystagmus. Tested by the Ishihara plates *there is no defect whatever of colour vision*. A marked degree of hemeralopia is present."

V. 79 is therefore night-blind, myopic but not colour-blind—an example of crossing-over.

V. 13. Karl P—k (1914). Research Chemist, Toronto. Tested for the writer in December 1939 by Dr J. W. MacArthur of Toronto University. Night-blind, myopic, but not colour-blind. Dr J. X. Robert of Toronto stated of him: "This patient has been under my care since March 1932 for the care of his eyes. At that time vision was as follows: R. -9 sph.,

—2 cyl., 105° ax.; Va 6/36; L. —6.5 sph., —2.5 cyl., 75° ax.; Va 6/36. Horizontal nystagmus when looking to extreme right or left. Examination of fundi showed discs inverted. Night-blindness was complained of at that time. Most recent examination 15 January 1940 showed no change in vision or refraction. Night-blindness and nystagmus continued as above. The field of vision is as shown on the enclosed chart" (i.e. Figs. 9, 10).

V. 22. David W—n (1924—). Birmingham. Incomplete red-green blind—the defect probably inherited from his mother. Tested in July 1939, he gave the Ishihara readings: 12, 8, 5, 70, 57, 2, 5, 17, 21, 2, 2, 2, 2, 2, 2, 2, 5, —, 45, 73, 28, 42, 85, 96.

VI. 26. Trevor G—y (1929—). Birmingham. Deuteranopic, but not night-blind or myopic. Tested in July 1939, he gave the Ishihara readings: 12, 8, 6, 70, 85, 2, 5, 17, 21, 2, 2, 2, 2, 2, 2, 2, 5, 2, 41, 21, 2, 4, 3, 9.

VI. 61. Brian M—e (1932—). Birmingham. Deuteranopic, but not night-blind or myopic. Tested in July 1939, he gave the Ishihara readings: 12, 3, 5, 70, 53, 2, 5, 11, 21, 2, 2, 2, 2, 2, 2, 2, 5, 8, 15, 73, 2, 4, 3, 9.

COMPILATION OF THE DATA

The technique of accumulating facts concerning the inheritance of human characteristics necessarily differs from case to case and, in fact, success depends not so much upon the capacities of the investigator as upon the attitude of the family concerned toward the research, and on the extent to which its members have maintained contact with one another. Given full co-operation by the family, the investigator has still to face up to the task of unearthing details long since submerged in the subconscious recollection and, worse still, has often to trace long-lost relatives with only a meagre amount of information to commence with. It is impossible to follow a standard procedure under such circumstances—much depends on the investigator's intuition—but an account of the manner in which the information given in the present work was obtained may be of some utility.

The present work commenced as the result of a visit by the writer to the home of Mr Paul I. W—n (VI. 18) during Whitsun 1939—a visit resulting from the fact that both the writer and VI. 18 were engaged upon chemical research in the same laboratory at the University of Birmingham. During the course of conversation, Mr J. W. W—n (V. 15), the father of VI. 18, mentioned that he suffered from night-blindness—

the accompanying severe myopia being an evident feature. While discussing possible physiological causes of the defect with the writer, V. 15 stated that it was present also in three of his four brothers and in several more distant relatives, all males. The compilation of a rough pedigree which followed this statement soon made it evident that the defect was sex-linked, and the writer, having some knowledge of genetics, decided to follow up the pedigree somewhat further than was possible with the information provided by V. 15 alone.

A further more significant detail soon appeared in consequence of a purely chance remark made by V. 15, who, referring to his work as an architect, stated that despite his myopia and night-blindness he had an excellent perception of colour tints and went on to remark: "In fact—what my wife says is blue very often isn't really blue at all."

Knowing that colour-blindness is sex-linked and is more frequent in males than in females, and feeling that Bell's (1932) statements regarding the non-association of sex-linked defects were founded on somewhat meagre evidence, the writer—a few days later—tested V. 15 for colour-blindness by means of the Ishihara charts and found that he was indisputably green-blind or deuteranopic. This discovery of the presence of sex-linked night-blindness and myopia together with deuteranopia made it imperative that the family pedigree should be followed up. Mr Paul I. W—n (VI. 18) readily agreed to help in the locating and visiting of such of his relatives as were available in the Birmingham district, and, in providing introductions to them and accompanying the writer on these visits, rendered invaluable service which alone made this research possible. In consequence of this help provided by VI. 18 all those members of the family who had to be seen co-operated readily—a feature which immediately overcame the major difficulty of this type of research, that of persuading members of a family to allow an investigator to enter their homes and ask numerous, sometimes perhaps unwelcome, questions.

Mr W. Clifford W—n (IV. 10) was first visited in order to obtain the addresses of members of the family whose location was unknown to V. 15 and VI. 18 and also to obtain data regarding the deceased members of earlier generations—a visit which resulted in the construction of a pedigree chart smaller than that illustrated, but containing most of its major details. The details of the chart were then filled in, checked and continually counter-checked, as the result of visits by the investigator to members of each of the branches of the family. All the information obtained was checked as carefully as possible by reference to documentary evidence (e.g. family bibles, marriage and birth certificates, census

records), and it can be said of the pedigree chart as illustrated, that the majority of its details, e.g. orders of birth, numbers of children, etc., are beyond dispute.

Having obtained this preliminary idea of the extent of the family, the writer commenced a systematic series of visits to as many of its members as possible—testing them all for colour-blindness and, where other eye defects were present, obtaining proper data concerning these from the oculists and specialists who had examined the eyes of the affected members. It is impossible here to specify the various ophthalmologists who thus aided the work, but the writer is more than grateful for the independent evidence they thus provided. On no occasion did the writer meet with any difficulty in obtaining their co-operation.

In the absence of any simple scientific apparatus for testing night-blindness it was necessary to rely upon statements from affected members as to whether or not they suffered from this defect. The ever-accompanying severe myopia, generally -10 to -14 diopters, made it easy, however, to locate such affected members, and in most of the cases it was necessary only to observe their behaviour under twilight conditions in order to confirm the presence of the night-blindness. Even in well-lit streets at night-time, such affected members had to be led by a normal person—a feature which the writer confirmed in most of the cases. In the cases of V. 4, V. 11, V. 15 and V. 22 the affected members were further tested for the writer by Mr Rudd, Senior Surgeon of the Birmingham and Midland Eye Hospital, as stated earlier. These tests confirmed the presence of night-blindness and severe myopia in all four cases, and also the presence of deuteranopia in the first three cases and its absence in V. 22. The deuteranopia in V. 4, 11 and 15 was confirmed by Mr Rudd by means of the Ishihara test, and they were also tested with the Edridge-Green lamp. The use of anything other than the largest apertures of this latter apparatus was, however, made impossible by the high myopia of the cases, and under such restricted conditions of test they were found to be capable of distinguishing between green and blue but *not* between red and purple, both of which were called red. The writer later found that several of the deuteranopic members confused not only green and blues but also red and purple, one of them (V. 46) confessing to a complete inability to recognize the latter colour.

So much for the manner in which proper confirmation was obtained concerning the presence or absence of the defects in various members of the family. The investigation proper commenced with visits to V. 11, V. 19 and V. 22, three of the four brothers of V. 15, the propositus. Of

these, V. 11 was found to be deuteranopic, night-blind and myopic, V. 19 was found to be deuteranopic only, and V. 22 was found to be night-blind myopic but not deuteranopic. It was immediately obvious that IV. 9—the mother of these four brothers—was capable of transmitting night-blindness, myopia and deuteranopia all on the same chromosome, and that to account for her two sons (V. 19 and V. 22) being only deuteranopic and night-blind myopic respectively, it was necessary to assume that crossing-over of the maternal XX sex-chromosomes had occurred during the process of formation of these two individuals. The real significance of the present work thus emerged at its very beginning.

The investigation was gradually extended until finally the writer had tested the majority of the members available in the Birmingham area, and several other cases of the occurrence of the defects were located, several members being discovered who were deuteranopic only and several who were night-blind myopic and deuteranopic.

In October 1939, the writer commenced certain other researches in London and was thus enabled to test V. 24, who was again found to have all three eye defects. Data for the descendants of V. 12 was obtained for the writer by Dr J. W. MacArthur of Toronto University, whose assistance in this respect was invaluable. The data regarding the descendants of IV. 15, IV. 18, III. 4 and III. 8 were, however, still incomplete, and the author was fortunately enabled to make several long journeys, to obtain the necessary data, by the Medical Research Council who financed the said journeys—an action for which the writer is extremely grateful. In the case of the descendants of IV. 15 and IV. 18, adequate data was obtained and IV. 41, the wife of IV. 15, provided the information regarding Mr Shurfly (I. 2), which was discussed earlier and was of importance in determining the source of the defects in the first few generations.

Most of the descendants of III. 4 were unfortunately found to have emigrated to America, and it was not found possible to locate their whereabouts or to determine their numbers with exactitude. In the case of the descendant of III. 8, the sole information available originally was a statement by IV. 16 that one of the sons, Walter Long (IV. 33), had worked on St Katherine's Dock, London, some sixty years ago. Further details of this branch of the family were lacking. A visit to St Katherine's Dock succeeded, after some tribulation, in bringing to light a foreman who had known IV. 33, and his information, followed by reference to the offices of the Port of London Authorities, provided details as to the date of death of IV. 33 (1931) and his address at that time.

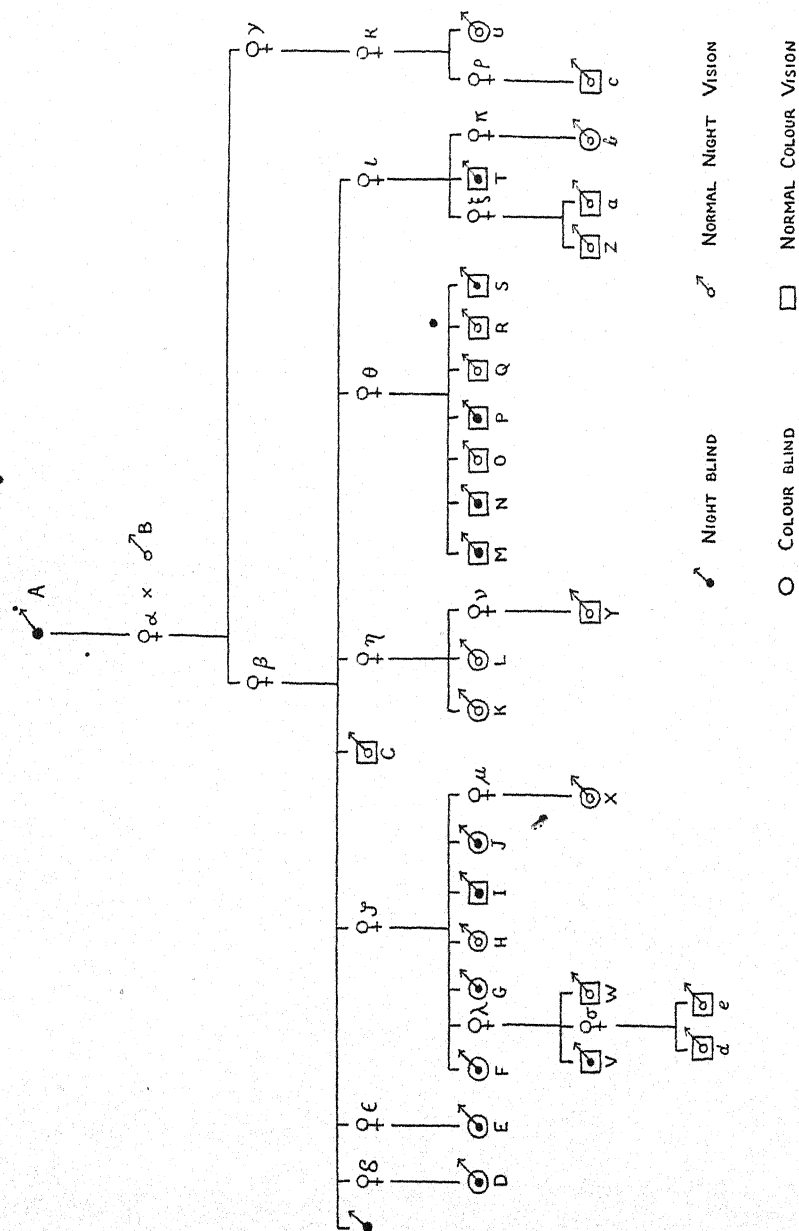
His wife (IV. 34) was found, on enquiry, to have left the said address, but by dint of much enquiry among her late neighbours, her present whereabouts were located and she was visited. In consequence it was found possible to locate and visit some of the children of IV. 33 and of his brother (IV. 39) and also to determine, with some reliability, the numbers of descendants of III. 8. Unfortunately, however, this section of the family appears to have lost touch with its immediate relatives, and it was not found possible to follow up its members as adequately as the rest of the pedigree. It can, however, be said with confidence as a result of what information was available, that this section of the family exhibits no night-blindness or myopia. If deuteranopia were present it would represent only one further chromosome cross-over, in the development of III. 8, so that the incompleteness of the information does not constitute a radical deficiency in the pedigree.

In the main, therefore, the pedigree is complete except that, as indicated earlier, full information is not available for two affected members who are known to have been night-blind myopic, but, being dead, cannot be stated to have been deuteranopic. A few other males who are dead or have emigrated may possibly be, or have been, deuteranopic, but the fact cannot be determined. It is certain, however, that all these male members of the pedigree who have been afflicted with night-blindness and myopia have been listed. The little extent to which the data is incomplete makes no difference to the fundamental conclusions of the work.

APPENDIX

I analyse the pedigree by the method of Bell & Haldane (1937). That is to say I assume the pedigree of night-blindness. Then, given the co-existence of night-blindness and colour-blindness in any one individual, I ask what is the probability $P(x)$, as a function of a recombination frequency x between the two genes, that the colour vision of all other relevant members of the pedigree was as found. I then calculate the value of x which makes the probability so found as large as possible.

I have summarized the information relevant to linkage in Fig. 11. Let x be the recombination frequency, and $y = 1 - x$. The gene for colour-blindness is symbolized by c , that for night-blindness and myopia by n . I have further made the simplifying assumption that there was only one source of deuteranopia in the family. Such an assumption may introduce a serious error when linkage is strong. For a given individual may either be due to a rare case of crossing-over or to a second source of



the disease. If the probabilities of crossing-over and of marrying a colour-blind person or one heterozygous for colour-blindness are of the same order of magnitude, the latter cannot be neglected. But where, as here, x is large, the correction is negligible.

The source of colour-blindness must have been α or β . In either case β was a double heterozygote, and δ , ϵ , ζ and ι , who are or were double heterozygotes, since both abnormalities appeared in their male progenies, must be $\frac{++}{c\ n}$.

Granted that G is cn , the probability of ζ being $\frac{++}{c\ n}$ is y . The further probability of F being cn is y . For given that he is n , the probabilities of his being $++$ and cn are x and y respectively. Similarly, the probabilities of H , I , and J being as found are x , x and y . Thus if F , G , H , I , J , were the only males investigated, the value of $P(x)$ would be x^2y^3 , and the likeliest value of x would be that which makes this quantity maximal, i.e. $\frac{2}{5}$, as might be expected. Next consider λ and her descendants. λ may have been $\frac{++}{++}$ or $\frac{++}{c\ n}$. The probabilities of these are respectively x and y . For since we take the pedigree of n as given, we neglect the possibilities that she was $\frac{++}{++}$ or $\frac{++}{c\ +}$. If λ was $\frac{++}{++}$ the probabilities of V , W , d and e being non-colour-blind are each unity. But if she was $\frac{++}{c\ n}$ the probabilities for V and W are x and y respectively.

And the probabilities of α being $\frac{++}{++}$, $\frac{++}{c\ +}$, $\frac{++}{+n}$, and $\frac{++}{c\ n}$ are $\frac{1}{2}y$, $\frac{1}{2}x$, $\frac{1}{2}x$, and $\frac{1}{2}y$ respectively. The corresponding probabilities that d should be $++$ are 1, $\frac{1}{2}$, 1, and y respectively, given that he is known not to be night-blind; and similarly for e . Hence the descendants of λ contribute a factor

$$x+y \cdot xy \left(\frac{1}{2}y + \frac{1}{2}x \cdot \frac{1}{4} + \frac{1}{2}x + \frac{1}{2}y \cdot y^2 \right)$$

to $P(x)$. This is

$$x + \frac{1}{8}xy^2 (5x + 4y + 4y^3),$$

or, since $x+y=1$,

$$\frac{1}{8}x (8x^5 + 40x^4y + 85x^3y^2 + 94x^2y^3 + 53xy^4 + 16y^5).$$

μ must be $\frac{++}{c\ +}$ or $\frac{++}{c\ n}$. The probabilities are $\frac{1}{2}x$ and $\frac{1}{2}y$. In the former case the probability of X being $c+$ is $\frac{1}{2}$, in the latter, x . Thus X contri-

432 Linkage and Crossing-over in Human Sex Chromosomes

butes a factor $\frac{1}{2}x \cdot \frac{1}{2} + \frac{1}{2}y \cdot x$, or $\frac{1}{4}x(x+3y)$. Thus if we only had knowledge of ζ 's male descendants, we should have

$$P(x) = 2^{-5}x^4y^3(x+3y)(8x^5+40x^4y+85x^2y^3+94x^2y^3+53xy^4+16y^5).$$

Here the term x^4y^3 corresponds to the four certain cases of crossing-over and three certain cases of non-crossing-over. The remaining terms represent possibilities. Putting $dP/dx=0$, we find $x=0.515$ approximately, whereas $P(x)=x^4y^3$ gives $x=0.571$. This is because the extra terms refer to possible cases of non-crossing-over. Thus in the descent of X from ζ crossing-over must have occurred in the oogenesis giving rise to μ or to X .

But if the latter hypothesis is correct, then μ was $\frac{++}{cn}$, i.e. arose from a non-cross-over gamete of ζ . So the genesis of X represents either one cross-over or one cross-over and one non-cross-over. The term $x(x+3y)$ symbolizes this fact.

In continuing the pedigree we must separately discuss two hypotheses. Given that ζ was $\frac{++}{cn}$, the probabilities that β was $\frac{++}{cn}$ and $\frac{+n}{c+}$ are y and x respectively.

(a) If β was $\frac{++}{cn}$ the probability that δ was $\frac{++}{cn}$ is y . The probability that, if so, D is cn is also y . Thus D contributes a factor y^2 , as does E , and C contributes a factor y . The probability of hypothesis (a) is thus far y^6 . η may be $\frac{++}{c+}$ or $\frac{+n}{cn}$, the probabilities being $\frac{1}{2}x$ and $\frac{1}{2}y$. In the first case the probabilities for K and L are each $\frac{1}{2}$, and that of Y , $\frac{3}{4}$. In the second case the probabilities for K and L are each x , while v may be $\frac{++}{++}$, $\frac{++}{c+}$, $\frac{+n}{+n}$, or $\frac{+n}{cn}$ with probabilities $\frac{1}{2}y$, $\frac{1}{2}x$, $\frac{1}{2}x$, and $\frac{1}{2}y$. The corresponding probabilities of Y being $++$ are 1 , $\frac{1}{2}$, 1 , and y . Thus if η is $\frac{++}{cn}$ the probability of Y is $\frac{1}{4}(3x^2+5xy+4y^2)$. Hence the descendants of η contribute a factor

$$\frac{1}{2}x \cdot \frac{3}{16} + \frac{1}{2}y \cdot \frac{1}{4}x^2(3x^2+5xy+4y^2)$$

or
$$2^{-5}x(3x^4+24x^3y+38x^2x^2+28xy^3+3y^4).$$

The probability that θ is $\frac{++}{cn}$ is y . If so the probabilities that M , N , P and S are $+n$ are each x , those that O , Q , and R are $++$ are each y . The probability that θ is $\frac{+n}{+n}$ is x . If so the probabilities for each of her

sons are unity. Thus her sons contribute a factor $y \cdot x^4y^3 + x$, or $x(1 + x^3y^4)$ to $P(x)$. Since T is night-blind and b colour-blind, ι is $\frac{++}{c\ n}$ with probability y . The possible genotypes of ξ are $\frac{++}{++}$, $\frac{++}{c\ +}$, $\frac{++}{+\ n}$, $\frac{++}{c\ n}$, with probabilities $\frac{1}{2}y$, $\frac{1}{2}x$, $\frac{1}{2}x$, and $\frac{1}{2}y$. The respective probabilities of Z and a being $++$ are 1, $\frac{1}{4}$, 1, and y^2 . Thus their contribution is

$$\frac{1}{2}y + \frac{1}{4} \cdot \frac{1}{2}x + \frac{1}{2}x + \frac{1}{2}y \cdot y^2 \quad \text{or} \quad \frac{1}{8} (5x^3 + 14x^2y + 13xy^2 + 8y^3).$$

T makes a contribution x , and b , like X , makes a contribution $\frac{1}{4}x(x+3y)$. Hence the probability of hypothesis (a) up to this point is

$$2^{-10}x^4y^7(x+3y)(3x^4+24x^3y+38x^2y^2+28xy^3+3y^4) \\ \times (5x^3+14x^2y+13xy^2+8y^3)(1+x^3y^4).$$

The last term can of course be made homogeneous by putting $(x+y)^7$ for 1.

We must now consider two more alternatives. Assuming that β was $\frac{++}{c\ n}$, B was $++$, and the probabilities that α was $\frac{++}{c\ n}$ and $\frac{++}{c\ +}$ are y and x .

(a₁) If α was $\frac{++}{c\ n}$, the probabilities of γ having been $\frac{++}{+c}$ and $\frac{++}{c\ n}$ are $\frac{1}{2}x$ and $\frac{1}{2}y$. Whence it follows that the same probabilities for κ are $\frac{1}{4}x(x+2y)$ and $\frac{1}{4}y^2$. If κ was $\frac{++}{c\ +}$ the probability that U should be $c+$ is $\frac{1}{2}$, that c should be $++$ is $\frac{3}{4}$. If κ was $\frac{++}{c\ n}$, the probability for U is x , that for c is $\frac{1}{4}(3x^2+5xy+4y^2)$ as in the case of Y . Hence the probability of hypothesis (a₁) is

$$2^{-5}xy(3x^4+15x^3y+33x^2y^2+31xy^3+14y^4).$$

(a₂) If α was $\frac{++}{c\ +}$, the probabilities of γ having been $\frac{++}{c\ +}$ and $\frac{++}{c\ n}$ are $\frac{1}{2}y$ and $\frac{1}{2}x$. The same probabilities for κ are $\frac{1}{4}(x^2+xy+y^2)$ and $\frac{1}{4}xy$. Hence the probability of hypothesis (a₂) is

$$x \left[\frac{1}{4}(x^2+xy+y^2) \cdot \frac{3}{8} + \frac{1}{4}xy \cdot x \cdot \frac{1}{4}(3x^2+5xy+4y^2) \right],$$

$$\text{or} \quad 2^{-5}x(3x^5+12x^4y+27x^3y^2+31x^2y^3+20xy^4+3y^5).$$

Thus the sum of the probabilities of hypotheses (a₁) and (a₂) is

$$2^{-5}x(3x^5+15x^4y+42x^3y^2+64x^2y^3+51xy^4+17y^5),$$

and the probability of hypothesis (a) is therefore

$$2^{-15}x^5y^7(x+3y)(5x^3+14x^2y+13x^2y^2+8y^3)(3x^4+24x^3y+38x^2y^2 \\ + 28xy^3+3y^4)(3x^5+15x^4y+42x^3y^2+64x^2y^3+51xy^4+17y^5)(1+x^3y^4).$$

434 Linkage and Crossing-over in Human Sex Chromosomes

(b) If β was $\frac{++}{c+}$, the probability that δ was $\frac{++}{c+}$ is x . Hence D contributes a factor xy . So does E , while C contributes a factor x . The probability of hypothesis (b) so far is x^4y^2 . η may be $\frac{++}{c+}$ or $\frac{++}{cn}$, the probabilities being $\frac{1}{2}y$ and $\frac{1}{2}x$. Hence, by the argument given above, her descendants contribute a factor

$$\frac{1}{2}y \cdot \frac{3}{16} + \frac{1}{2}x \cdot \frac{1}{4}x^2 (3x^2 + 5xy + 4y^2)$$

or $2^{-5} (12x^5 + 23x^4y + 28x^3y^2 + 18x^2y^3 + 12xy^4 + 3y^5)$.

θ was $\frac{++}{c+}$ with probability y , or $\frac{++}{cn}$ with probability x . Hence her sons contribute a factor $y + x^4y^3x$, or $y(1 + x^5y^2)$. ι was $\frac{++}{cn}$ with probability x . Hence her male descendants contribute a factor

$$2^{-5}x^3 (x + 3y) (5x^3 + 14x^2y + 13xy^2 + 8y^3)$$

by the argument given above. Thus the probability of hypothesis (b) so far is

$$2^{-10}x^7y^3 (x + 3y) (5x^3 + 14x^2y + 13xy^2 + 8y^3) (12x^5 + 23x^4y + 28x^3y^2 + 18x^2y^3 + 12xy^4 + 3y^5) (1 + x^5y^2).$$

Given that β was $\frac{++}{c+}$, it follows that α was $\frac{++}{c+}$ and B was $c+$.

Hence the probabilities that γ was $\frac{++}{c+}$ and $\frac{++}{cn}$ are each $\frac{1}{2}$. The probabilities that κ was $\frac{++}{c+}$ and $\frac{++}{cn}$ are $\frac{1}{2}(x + 2y)$ and $\frac{1}{2}x$. If κ was $\frac{++}{c+}$ the probability that U should be $c+$ is $\frac{1}{2}$, the probability that c should be $++$ is $\frac{3}{4}$. If κ was $\frac{++}{cn}$ these probabilities are x and $\frac{1}{4}(3x^2 + 5xy + 4y^2)$, as above. Hence the contribution of U and c to the probability of hypothesis (b) is a factor

$$2^{-4} (9x^4 + 25x^3y + 35x^2y^2 + 21xy^3 + 6y^4).$$

Hence the probability of this hypothesis is

$$2^{-14}x^7y^3 (x + 3y) (5x^3 + 14x^2y + 13xy^2 + 8y^3) (9x^4 + 25x^3y + 35x^2y^2 + 21xy^3 + 6y^4) (12x^5 + 23x^4y + 28x^3y^2 + 18x^2y^3 + 12xy^4 + 3y^5) (1 + x^5y^2).$$

Hence the sum of the probabilities of hypotheses (a) and (b) is

$$2^{-15}x^5y^3 (x + 3y) (5x^3 + 14x^2y + 13xy^2 + 8y^3) [y^4 (3x^4 + 24x^3y + 38x^2y^2 + 28xy^3 + 3y^4) (3x^5 + 15x^4y + 42x^3y^2 + 64x^2y^3 + 51xy^4 + 17y^5) (1 + x^3y^4) + 2x^2 (x + y)^2 (9x^4 + 25x^3y + 35x^2y^2 + 21xy^3 + 6y^4) (12x^5 + 23x^4y + 28x^3y^2 + 18x^2y^3 + 12xy^4 + 3y^5) (1 + x^5y^2)].$$

This expression is complicated by the terms $(1+x^3y^4)$ and $(1+x^5y^2)$. These are the expressions of the probability, which is certainly less than 2^{-7} , that θ was heterozygous for colour-blindness, yet none of her seven sons showed it. This probability is far less than that of a second source of colour-blindness, which we have neglected already. And it will not have a great influence one way or the other, on the value of x . We shall therefore neglect it. The expression in the square bracket now becomes

$$[216x^{13} + 1,446x^{12}y + 4,738x^{11}y^2 + 9,840x^{10}y^3 + 14,356x^9y^4 + 15,693x^8y^5 \\ + 13,600x^7y^6 + 10,314x^6y^7 + 7,926x^5y^8 + 6,434x^4y^9 + 4,706x^3y^{10} \\ + 2,302x^2y^{11} + 629xy^{12} + 51y^{13}].$$

$$\text{So } P(x) = 2^{-20}x^9y^6(x+3y)^2(5x^3+14x^2y+13xy^2+8y^3)(8x^5+40x^4y \\ + 85x^3y^2+94x^2y^3+53xy^4+16y^5)(216x^{13}+1,446x^{12}y+4,738x^{11}y^2 \\ + 9,840x^{10}y^3+14,356x^9y^4+15,693x^8y^5+13,600x^7y^6+10,314x^6y^7 \\ + 7,926x^5y^8+6,434x^4y^9+4,706x^3y^{10}+2,302x^2y^{11}+629xy^{12}+51y^{13}).$$

To simplify this expression we put $x = \frac{1}{2}(1+z)$, and find

$$P(z) = P(x) = 2^{-46}(1+z)^9(1-z)^6(2-z)^2(10-2z+3z^2-z^3) \\ (74-22z+17z^2-9z^3+5z^4-z^5)(1,440+2,124z+8,849z^2+3,114z^3 \\ + 6,641z^4+3,390z^5-400z^6+1,666z^7-216z^8+254z^9-25z^{10}+12z^{11}-z^{12}).$$

This has its maximum value when

$$\frac{d}{dz} \log P(z) = \frac{9}{1+z} - \frac{6}{1-z} - \frac{2}{2-z} - \frac{2-6z+3z^2}{10-2z+3z^2-z^3} \\ - \frac{22-34z+27z^2-20z^3+5z^4}{74-22z+17z^2-9z^3+5z^4-z^5} + 2(1,026+8,849z \\ + 4,671z^3+13,282z^3+8,475z^4-1,200z^5+5,831z^6+864z^7+1,143z^8 \\ - 125z^9+66z^{10}-6z^{11}) \div (1,440+2,124z+8,849z^2+3,114z^3+6,641z^4 \\ + 3,390z^5-400z^6+1,666z^7-216z^8+254z^9-25z^{10}+12z^{11}-z^{12}) = 0.$$

This is an equation of the 22nd degree, and it is best not to expand it, but to calculate the value of the left-hand side for different values of z , and interpolate. The only root between ± 1 , is $z = 0.2956$, hence $x = 0.648$.

The amount of information concerning z is obtained by substituting this value in $-d^2/dz^2 \log P(z)$. It is 15.57 units. Hence the amount of information concerning x is 62.27 units, and the standard error of x is 0.127. Thus the estimated cross-over value is $64.8 \pm 12.7\%$.

The true value is presumably under 50%, but it is unlikely to be under 40%. Thus the genetical map of the human X-chromosome will be fairly long, and it was an accident that the first pair of genes studied in it, namely those for colour-blindness and haemophilia, turned out to be fairly closely linked.

436 *Linkage and Crossing-over in Human Sex Chromosomes*

It is to be noted that if two autosomal genes had shown as great independence as those here considered, they might well have been assigned to different chromosomes. If other mammals have a similar linkage system it is quite possible that genes now regarded as independent may ultimately be assigned to the same linkage group. It seems, moreover, that the chances of finding significant linkage between two autosomal genes chosen at random is somewhat less than had been hoped.

SUMMARY

Previous knowledge regarding the inheritance of sex-linked congenital stationary night-blindness, myopia, and deuteranopia is discussed, and the necessity of obtaining information concerning the linkage of human characteristics and the crossing-over of human sex-chromosomes is indicated.

A pedigree is described in which the transmission of associated night-blindness, myopia, and deuteranopia through seven generations of a family is exhibited. The mode of transmission of the defects shows that they are sex-linked and were originally all located on one X-chromosome but have, on several occasions, been separated by crossing-over of the maternal X-chromosomes. An estimate of the cross-over ratio between the genes for night-blindness (with myopia) and deuteranopia in human beings is given, enabling the distance between these genes to be determined.

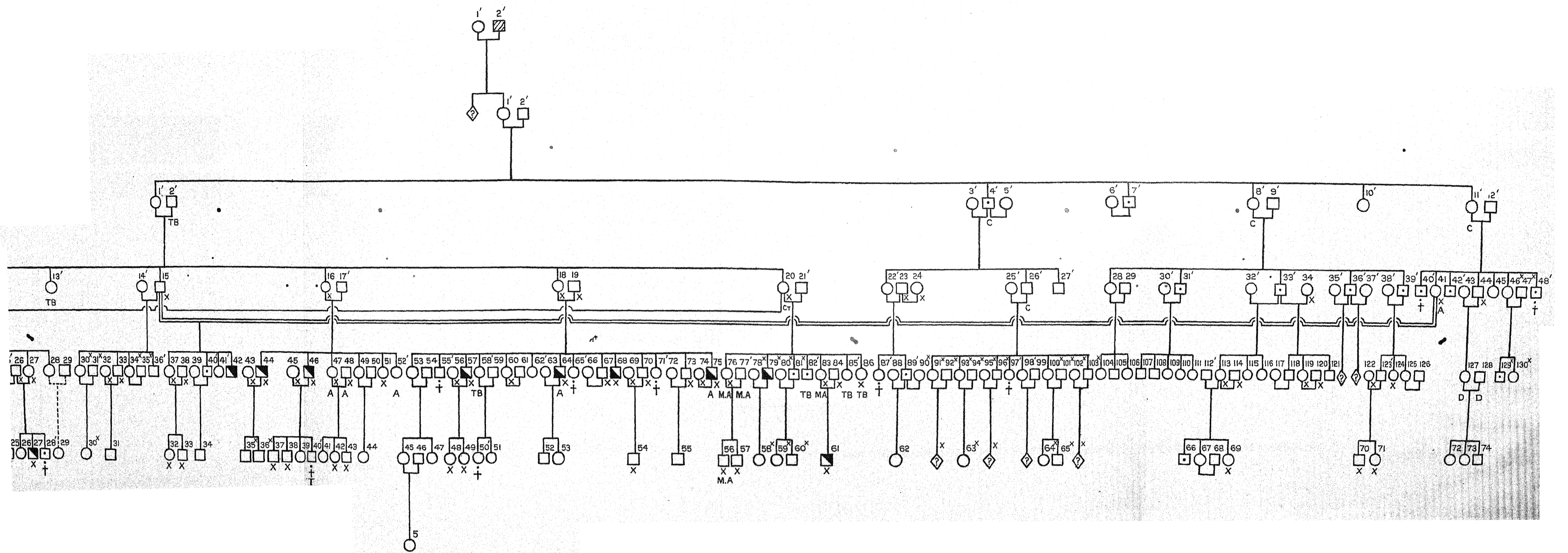
Details are given regarding the affected individuals and the method of compiling the information. A Statistical Appendix by Prof. J. B. S. Haldane, F.R.S., gives a full mathematical treatment of the data.

Finally, the author wishes to express his indebtedness and gratitude to all those members of the family who have co-operated so sincerely in making this work possible, and particularly to Paul I. Wilson (VI. 18) for his invaluable assistance in helping the writer to make contact with the members of the family. The author is much indebted also to the Medical Research Council for a grant which enabled the completion of the work; to Mr Rudd for the invaluable aid described earlier; to Dr J. W. MacArthur of Toronto University for testing sundry members located in Toronto; to numerous ophthalmologists; and to Prof. J. B. S. Haldane, F.R.S., for his help, criticism and encouragement of this work.

REFERENCES

- BELL (1932). *Treasury of Human Inheritance*, 2.
- BELL & HALDANE (1937). *Proc. roy. Soc. B*, **123**, 119.
- FISHER (1936). *Ann. Eugen., Lond.*, **7**, 87.





436 *Linkage and Crossing-over in Human Sex Chromosomes*

It is to be noted that if two autosomal genes had shown as great independence as those here considered, they might well have been assigned to different chromosomes. If other mammals have a similar linkage system it is quite possible that genes now regarded as independent may ultimately be assigned to the same linkage group. It seems, moreover, that the chances of finding significant linkage between two autosomal genes chosen at random is somewhat less than had been hoped.

SUMMARY

Previous knowledge regarding the inheritance of sex-linked congenital stationary night-blindness, myopia, and deuteranopia is discussed, and the necessity of obtaining information concerning the linkage of human characteristics and the crossing-over of human sex-chromosomes is indicated.

A pedigree is described in which the transmission of associated night-blindness, myopia, and deuteranopia through seven generations of a family is exhibited. The mode of transmission of the defects shows that they are sex-linked and were originally all located on one X-chromosome but have, on several occasions, been separated by crossing-over of the maternal X-chromosomes. An estimate of the cross-over ratio between the genes for night-blindness (with myopia) and deuteranopia in human beings is given, enabling the distance between these genes to be determined.

Details are given regarding the affected individuals and the method of compiling the information. A Statistical Appendix by Prof. J. B. S. Haldane, F.R.S., gives a full mathematical treatment of the data.

Finally, the author wishes to express his indebtedness and gratitude to all those members of the family who have co-operated so sincerely in making this work possible, and particularly to Paul I. Wilson (VI. 18) for his invaluable assistance in helping the writer to make contact with the members of the family. The author is much indebted also to the Medical Research Council for a grant which enabled the completion of the work; to Mr Rudd for the invaluable aid described earlier; to Dr J. W. MacArthur of Toronto University for testing sundry members located in Toronto; to numerous ophthalmologists; and to Prof. J. B. S. Haldane, F.R.S., for his help, criticism and encouragement of this work.

REFERENCES

- BELL (1932). *Treasury of Human Inheritance*, 2.
BELL & HALDANE (1937). *Proc. roy. Soc. B*, **123**, 119.
FISHER (1936). *Ann. Eugen., Lond.*, **7**, 87.

- HALDANE (1936). *Ann. Eugen., Lond.*, **7**, 28.
 MADLENER (1928). *Arch. Rass.- u. GesBiol.* **20**, 390.
 PUNNETT (1933). *Trans. ophthal. Soc. U.K.* **53**, 9.
 RATH (1938). *Arch. Rass.- u. GesBiol.* **32**, 397.
 RIDDELL (1937). *Brit. J. Ophthal.* **21**, 113.

KEY TO PEDIGREE SYMBOLS

- Male. Normal vision.
 ■ Night-blind, myopic, colour-blind.
 ◼ Night-blind, myopic, normal colour vision.
 ◼ Colour-blind, normal night vision.
 ▨ Night-blind, myopic, colour perception not tested.
 ◻ Normal night vision, colour perception not tested.
 ▩ Colour-blindness derived from extra-familial source.
 ○ Female.
 □ ? □ Twins, not known if identical.
 □ Consanguinity.
 - - - Illegitimate.
 ◇ Sex and number unknown.
 I-VII Generation.
 1-130 Reference number.
 1' Dead.
 1× Emigrated.
 † Died in infancy.
 ↗ Propositus.
 □ × Tested for eye defects.
 M Myopic.
 A Astigmatic.
 C Cancer.
 CT Cataract.
 D Deaf mute.
 I Insanity.
 N Nystagmus.
 TB Tuberculosis.



NOTE ON THE INHERITANCE OF YELLOW BILL COLOUR IN DUCKS

By J. M. RENDEL

NORMALLY the bills of Aylesbury ducks are of a pale flesh colour, but the exact colour is very variable. The bill of the mature drake is usually more yellow than that of the duck and there is much less yellow in the duck's bill when she is in full lay than before she starts laying. Occasionally a duck with a bright orange-yellow bill is found. This bright yellow colour, which is typical of such breeds as the White Campbell and the Pekin, will also fade during the laying season, but it is always distinguishable from the normal. When the birds are young the difference is unmistakable.

During an experiment carried out at the National Institute of Poultry Husbandry, and reported elsewhere, records were kept of the bill colour of parents and their offspring. The parents were of two kinds. Six pens, each containing one drake and four ducks, belonged to a breed produced by crossing Aylesbury and White Campbell. Six similar pens contained pure Aylesburys. The former are shown in the table as Allports, the latter as N.P.I. In pen 9, one duck died and has not been recorded.

Amongst the parent birds used in this experiment, one duck (no. 219) had a bright yellow bill, but produced no yellow-billed offspring, nor did any of the birds in the same pen with her. Thirteen of the other forty-three matings, however, did produce yellow-billed offspring. From these thirteen matings 276 ducklings were reared, of which 210 were normal and 66 had yellow bills. This fits very closely the 3 : 1 ratio which one would expect, if the character were an autosomal recessive, the expected numbers being 207 and 69. The families are uniform as regards the number of yellow-billed compared to normal birds occurring in each family, since $\chi^2 = 12.32$ for 12 degrees of freedom; the number of families showing yellow bill is large compared to the frequency of yellow-billed parents. This reflects the considerable amount of inbreeding and of selection against yellow bills in both strains of duck used. The evidence shows that yellow bill is inherited as an autosomal recessive.

440 *Note on the Inheritance of Yellow Bill Colour in Ducks*

Inheritance of yellow bill colour

All-ports Dam	Families with no yellow		Families with yellow				N.P.I. Dam	Families with no yellow		Families with yellow			
			Normal		Yellow					Normal		Yellow	
	M.	F.	M.	F.	M.	F.		M.	F.	M.	F.	M.	F.
Pen 1:							Pen 7:						
219	9	4	—	—	—	—	248	15	14	—	—	—	—
235	9	3	—	—	—	—	252	4	5	—	—	—	—
409	10	11	—	—	—	—	78	6	8	—	—	—	—
413	16	10	—	—	—	—	82	—	—	4	1	1	2
Pen 2:							Pen 8:						
215	3	9	—	—	—	—	261	—	—	11	8	1	3
216	18	18	—	—	—	—	276	—	—	5	15	2	4
415	—	—	12	13	2	3	71	12	10	—	—	—	—
416	—	—	12	4	1	4	80	15	27	—	—	—	—
Pen 3:							Pen 9:						
208	14	9	—	—	—	—	283	15	12	—	—	—	—
209	9	11	—	—	—	—	74	5	3	—	—	—	—
211	9	15	—	—	—	—	76	17	12	—	—	—	—
410	—	—	3	7	1	2							
Pen 4:							Pen 10:						
221	20	15	—	—	—	—	280	—	—	7	11	1	2
329	15	15	—	—	—	—	282	—	—	5	11	3	2
332	12	12	—	—	—	—	77	15	21	—	—	—	—
412	18	10	—	—	—	—	81	11	9	—	—	—	—
Pen 5:							Pen 11:						
210	3	5	—	—	—	—	257	—	—	3	2	5	2
238	3	12	—	—	—	—	266	—	—	13	12	6	3
408	11	12	—	—	—	—	72	13	10	—	—	—	—
414	21	18	—	—	—	—	73	—	—	15	12	4	7
Pen 6:							Pen 12:						
213	8	5	—	—	—	—	260	11	12	—	—	—	—
258	13	8	—	—	—	—	279	11	14	—	—	—	—
331	—	—	7	6	3	0	75	9	17	—	—	—	—
411	—	—	5	6	1	1	79	13	13	—	—	—	—
Total	221	202	39	36	8	10	Total	172	187	63	72	23	25
					Yellow	Normal					Yellow	Normal	
	No. found				18	75		No. found			48	135	
	No. expected				23·25	69·75		No. expected			45·75	137·25	
	Difference				5·25			Difference			2·25		
	Standard error				4·18			Standard error			5·86		

CYTOGENETICS OF *BRASSICA* HYBRIDS AND SPECIES¹

By S. M. SIKKA, *Dept. of Agriculture, Lyallpur, India*

(With Plates XVI-XX, containing Figs. 1-153, and
Three Diagrams in the Text)

CONTENTS

	PAGE
1. Introduction	441
2. Material	444
3. Cytological technique	445
4. Observations	447
Part I. <i>Brassica</i> hybrids:	
(i) <i>Brassica juncea</i> Coss. × <i>B. campestris</i> L. var. <i>sarson</i> Prain	447
(ii) <i>Brassica Tournefortii</i> Gouan × <i>B. trilobularis</i> H.f.T.	456
(iii) <i>Brassica trilobularis</i> H.f.T. × <i>B. rapa</i> L.	465
Part II. <i>Brassica</i> species:	
A. Somatic chromosomes	466
B. Meiosis	473
5. Discussion	488
Species formation in <i>Brassica</i>	488
6. Summary	496
7. Acknowledgements	498
References	498
Explanation of Plates XVI-XX	506

1. INTRODUCTION

THE genus *Brassica* includes about one hundred species, the majority of which are native to the Mediterranean region. A number of them, however, are now in cultivation throughout the world, especially in India and Japan, where they rank as important oilseed crops.

The cytological work on this genus has so far been confined to cultivated species, and a vast majority of wild forms has remained unstudied. The various species which have been worked with so far can be classified into the following eight groups on the basis of their haploid chromosome numbers (Table I). For the chromosome numbers of these species, excepting *B. sinapistrum*, *B. Tournefortii*, *B. monensis* and *B. rugosa*, chromosome numbers of which are here determined for the first time, and also *B. cheiranthus* and *B. Wrightii*, the numbers of which were found by Gates (quoted by Wright, 1936), the lists given by Karpechenko (1922), Shimotomai (1925), and Nagai & Sasaoka (1930) have been consulted.

¹ Part I of thesis approved for the Ph.D. degree of the University of London.

It will be noticed from Table I that the whole genus is characterized by aneuploid numbers of chromosomes. This remarkable prevalence of aneuploidy throughout the genus makes it a particularly interesting group for study, both with a view to chromosome evolution and to the difficult taxonomic problem of fixing specific boundaries. During the past few years attempts have been made by several investigators to analyse the intricate species relationships in this genus, the various methods of approach to the problem being: (i) study of the morphology of somatic chromosomes, (ii) study of secondary association of chromosomes at meiosis, (iii) analysis of the genomic constitution of different species as revealed from the study of interspecific and intergeneric hybrids, (iv) study of haploid plants which arose spontaneously in certain species.

TABLE I

Group		Species
I	n=8	<i>B. nigra</i>
II	n=9	<i>B. oleracea</i> , <i>B. alboglabra</i> , <i>B. sinapis</i>
III	n=10	<i>B. campestris</i> , <i>B. rapa</i> , <i>B. chinensis</i> , <i>B. japonica</i> , <i>B. pekinensis</i> , <i>B. trilobularis</i> , <i>B. Tournefortii</i>
IV	n=12	<i>B. Wrightii</i> , <i>B. monensis</i>
V	n=17	<i>B. carinata</i>
VI	n=18	<i>B. juncea</i> , <i>B. cernua</i>
VII	n=19	<i>B. napus</i> , <i>B. napella</i> , <i>B. rugosa</i>
VIII	n=24	<i>B. cheiranthus</i>

Catcheside (1934) has analysed the morphological differences between the somatic chromosomes of *B. napus* and *B. rapa*. He found that more or less similar chromosome types occurred in these two species, although the frequency of the types was different in each. Results of a similar kind of study made by Alam (1936) in *B. campestris*, *B. trilobularis* and *B. juncea* and by Richharia (1937) in *B. oleracea*, *B. pekinensis*, *B. rapa* and *B. chinensis* also point in the same direction. However, this type of study in *Brassica* has not so far furnished any conclusive clues to the phylogeny of various species, as the task has been very much hampered by difficulties arising from the extremely small size of the chromosomes, which make minute observations impossible.

From the secondary association of chromosomes Catcheside (1934) and Alam (1936) came to the conclusion that the species studied by them are secondarily balanced polyploids on the primary basic number of six. Richharia (1937b), however, could not arrive at any definite conclusion with regard to the primary basic number for the genus, due to a large degree of association between morphologically different chromosomes, which suggested some sort of chromosome rearrangements, besides re-duplication.

Observations on chromosome conjugation in interspecific and intergeneric hybrids raised by several investigators has been the most significant method of approach to the study of phylogeny and the complex problem of species formation in *Brassica*, as in other genera like *Triticum*, *Gossypium*, *Nicotiana*, etc.

Hybridization in *Brassica* has been tried since 1834, when Herbert (quoted by Focke, 1881) succeeded in raising the F_1 between *B. napus* and *B. rapa*. At the same time, Sagaret succeeded in crossing *B. oleracea* with *B. rapa* and *B. napus*, when the former was used as a male parent. As to more recent attempts, one may refer to Fruwirth's *Handbuch der landwirtschaftlichen Pflanzenzüchtung*, Bd. 2 (1924). Extensive genosystematical investigations of cultivated *Brassica* published by Sinskaia (1927) also give a general survey of the interspecific crossability in *Brassica*. It was, however, not until 1928 that any serious cytogenetic studies were made in interspecific hybrids of *Brassica*, and the credit of doing so for the first time must be given to Morinaga (1928, 1929, 1931, 1933, 1934). His work, followed by that of Sasaoka (1930), U (1935) and Karpechenko (1937), has shown that the species with higher chromosome numbers (e.g. *B. carinata*, *B. cernua*, *B. napus*) can be taken to be allotetraploids whose genomes are composed of two of those found in the three species with lower chromosome numbers, viz. $n=8$, 9 and 10.

Richharia (1937b) studied three crosses between twenty chromosomal species of *Brassica* and observed complete conjugation of chromosomes in each of them, excepting *B. pekinensis* \times *B. rapa*, where two free univalents were occasionally found at metaphase I.

The most recent work of the Russian school in obtaining tetraploid cabbage by "apical regeneration" of a decapitated shoot of ordinary cabbage (Schavinskaya, 1937) which increased its crossability with other species (Karpechenko, 1937a) and made the raising of hexagenomic and octogenomic hybrids possible, has been another source of evidence in revealing the nature of the genomes, of which *Brassica* species are constituted. Karpechenko (1937b) studied the meiosis of F_1 cross between tetraploid cabbage ($n=18$) and *B. carinata* ($n=17$) and observed the formation of trivalents at metaphase I due to pairing of cabbage chromosomes with the corresponding nine chromosomes from the mustard set. By doubling artificially the chromosomes of the F_1 hybrid he obtained an octogenomic hybrid containing four sets from cabbage and two sets from mustard, the reduction division of which showed formation of various multivalents up to sexivalents in the form of both rings and chains. Some of these multivalents were undoubtedly formed allo-

syndetically by the union of homologous sets of chromosomes present both in cabbage and mustard.

The study of intergeneric crosses, though not directly bearing on the question of genome analysis in *Brassica*, has indirectly proved fruitful in confirming the results obtained from interspecific hybrids. The results of the intergeneric cross made by Karpechenko (1927, 1928) between *Raphanus* and *Brassica oleracea* and the origin of the amphidiploid *Raphanobrassica* (RRBB) in the progeny of this cross are well known. Karpechenko (1929) analysed cytologically the hybrids between *Raphanobrassica* and *Brassica carinata* and observed that nine bivalents were invariably formed in the F_1 due to the conjugation of nine homologous chromosomes contributed by each of the parents, which is quite in conformity with the behaviour of the interspecific cross *B. oleracea* \times *B. carinata*.

The only haploid plant so far known in *Brassica* is that of *B. napella* which arose spontaneously in the cultures of the latter species. Morinaga & Fukushima (1933) studied the meiosis of this haploid plant and observed the formation of 0-6 and sometimes 7 bivalents at metaphase I.

Although a lot of useful work has been done during the past few years towards solving the difficult question of species formation in *Brassica*, yet a number of important problems still remain untackled. For instance, how the so-called "mongenomic" species, by the crossing and subsequent doubling of which the digenomic species have arisen, have themselves originated, is not known. Again, the study of the satellites in relation to the nucleolus, which with the brilliant discoveries of Heitz and McClintock has become of increasing interest, has been totally ignored in this genus. Furthermore, Gates (1937) has shown how the study of satellites could be employed also in revealing the phylogeny of the nucleus. It was with the object of collecting data on some of these problems that the present work, which deals with the cytology of some species and hybrids not hitherto studied, was undertaken.

2. MATERIAL

The following pure species and hybrids were under investigation:

A. Species:

- (1) *B. nigra* Koch.
- (2) *B. oleracea* L.
- (3) *B. rapa* L.
- (4) *B. campestris* L., var. *sarson* Prain.
- (5) *B. trilocularis* H.f.T.

- (6) *B. Tournefortii* Gouan.
- (7) *B. juncea* Coss.
- (8) *B. rugosa* L.
- (9) *B. monensis* Huds.
- (10) *B. Sinapistrum* Boiss.
- (11) *B. napus* L.
- (12) *B. Wrightii*.

B. Hybrids:

- (1) *B. trilocularis* \times *B. Tournefortii*.
- (2) *B. juncea* \times *B. campestris*.
- (3) *B. rapa* \times *B. trilocularis*.

The seeds of species number (1)–(7) were obtained from K. S. Ali Mohammad, Botanist for Oilseeds, Department of Agriculture, Punjab, Lyallpur, and I take this opportunity of thanking him for the trouble he took in providing this supply. My thanks are also due to Prof. R. R. Gates for obtaining seeds of species numbers (9) and (10) from the Isle of Man, and of number (11) from the Director, Royal Botanic Gardens, Kew. The cytological material of *B. Wrightii* was collected from plants grown by Prof. Gates from the seeds originally obtained by him from Dr Ellison Wright.

The crosses were made by the author in the botanical section of the Agricultural Research Institute, Lyallpur, during January 1936.

For the study of somatic chromosomes, actively growing root-tips were obtained from seeds germinated on moist filter paper or pure sand. The latter gave healthier and much more straight root-tips, which contained a larger number of dividing cells than those germinated on filter paper. For the study of meiotic chromosomes, flower buds were obtained from plants grown at the Courtauld Genetical Laboratory, Regent's Park, during the summer of 1938. The seeds of all the species and crosses were sown in pots inside the greenhouse as well as out in the field. The plants in the field remained very stunted due to unfavourable weather conditions, whereas the plants in the greenhouse made quite satisfactory growth and provided enough material for cytological studies.

3. CYTOLOGICAL TECHNIQUE

The root-tips were fixed between 11.0 a.m. and 12.0 noon, in Nava-shin's chrom-acetic formalin, Benda's solution with low acetic acid and Lewitsky's chromic formalin (1 : 1) for about 24 hr. A little maltose was added to each of the fixatives used before actually immersing the root-

tips, as the addition of maltose was found to spread the metaphase plates well. An exhaust pump was invariably used. The root-tips were then thoroughly washed in tepid water, graded up through series of alcohol and chloroform and embedded in paraffin. Sections were cut 10–12 μ thick.

All the three fixatives used gave quite good fixation, but Lewitsky's proved to be the best, since it gave long, slender chromosomes in which satellites could be made out clearly. With no other fixative could the satellites be studied. But with Lewitsky's fixative the chromosomes could not be stained brightly with gentian violet. To get over this trouble the sections were stained in decolorized fuchsin prepared according to the schedule given in Bolles Lee, *Microtomists' Vade Mecum* (1937).

For determining the number of nucleoli in somatic telophases the slides stained in decolorized fuchsin were counterstained in saturated solution of fast green in 70 % alcohol, in accordance with the schedule given by Semmens & Bhaduri (1939), with the modification that the slides were not mordanted in sodium carbonate solution prior to counterstaining, since previous mordanting in alkali definitely hindered the staining of the nucleolus in this material. For the proper differentiation of chromosomes, cytoplasm and nucleolus, a very weak solution of sodium carbonate in 80 % alcohol was used. Lewitsky's fixative, again, proved to be the best for obtaining brightly stained nucleoli. By the use of this technique, which resulted in differential staining of the chromosomes (violet) and nucleolus (green), the task of determining the number of nucleoli was rendered very easy. By gentian violet staining, which stains the chromosomes and nucleolus alike, such a task could have been completed only with great labour and difficulty.

Flower buds were fixed overnight in Navashin's solution and medium Flemming with good results. Before actually immersing the buds in the fixative the perianth was removed as far as possible, and anthers dipped in Carnoy's solution (Semmens' modification, 1937) for 2–3 sec., followed by thorough rinsing in water. An exhaust pump was invariably used for sinking the buds in the fixative. Acetocarmine was used for determining the stage of division in the pollen mother cells. The material was embedded and sectioned as in the case of root-tips. For staining, Newton's iodine-gentian-violet technique was invariably followed. Owing to the extremely small size of meiotic chromosomes, Feulgen staining was not very successful.

4. OBSERVATIONS

PART I. *BRASSICA* HYBRIDS

- (i) *Brassica juncea* Coss. ($2n=36$) \times *B. campestris* (L. var. sarson *Prain* ($2n=20$))

Twenty-nine buds of *B. juncea* were pollinated with pollen of *B. campestris* and twenty-one pods containing thirty-two seeds in all were obtained. The F_1 seeds, though much smaller in size than those of either of the parents, gave cent per cent germination. Table II gives the comparative characters of the parental species and their F_1 hybrids.

TABLE II

	<i>B. juncea</i>	<i>B. campestris</i>	F_1 hybrid
Leaves	Pale green, glabrous, petio- late	Dark green, pubescent, amplexicaul	Dark green, pubescent, slightly petioled
Flowers	Yellow, smaller in size with petals not overlapping	Bright yellow, large, with slightly overlapping petals	Bright yellow, inter- mediate in size
Anthers	Introrse	Extrorse	Extrorse
Pods	Short and torulose	Long, cylindrical	Intermediate
Seeds	Brown	Brown	Brown

A. *Cytology of the parent species.*

(i) *Brassica juncea* Coss. ($2n=36$). The somatic chromosomes of this species will be described in Part II of this paper. Here its meiotic behaviour only will be dealt with.

At diakinesis generally eighteen bivalents are formed in the nucleus (Fig. 1). In certain cases three bivalents were observed to lie on the single nucleolus present at this stage (Fig. 2). These would correspond to six satellitic chromosomes observed in the somatic complement. Among the bivalents, two types—rings and rods—can be made out, the variation of size in each of them being quite marked. Occasionally one bivalent is replaced by two univalent chromosomes (Fig. 3). These univalents most probably result from failure of chiasma formation in the shortest pair of chromosomes in the complement. Very rarely a nucleus shows a ring of four chromosomes besides other bivalents (Fig. 4). If one or two chiasmata fail in the ring, a chain of four chromosomes or a trivalent and a univalent would result. Both these have been observed and are illustrated in Figs. 2 and 3, respectively. On the whole, the frequency of quadrivalent formation is very low, these having been found in less than 3 % of cells; exact statistics are difficult to obtain, since the chromosomes being very small it is easier to observe a quadrivalent at diakinesis than to count eighteen bivalents and so prove its absence.

From the results of interspecific hybridization, as well as study of somatic chromosomes, it will be shown later in this paper that *B. juncea* is an amphidiploid derivative of the cross *B. campestris* \times *B. nigra*. On this account, therefore, this species is expected to show only bivalent formation at meiosis, as is the characteristic of amphidiploids. But since it shows some frequency of quadrivalents at diakinesis, it seems logical to infer that the species from the cross of which *B. juncea* arose by doubling of chromosomes are nearly related and have some chromosomes in common.

Several other cases of amphidiploid species are known where quadrivalent formation occurs to a greater or lesser degree. Examples are *Primula kewensis* (Newton & Pellew, 1929), *Nicotiana tabacum-sylvestris* (Rybin, 1929), *Nicotiana rustica-paniculata* (Lammerts, 1931) and *Crepis rubra-foetida* (Poole, 1931). In the last amphidiploid all the chromosomes sometimes appear in the form of quadrivalents.

Fig. 5 is a polar view metaphase showing eighteen bivalents, while Fig. 6 illustrates seventeen bivalents and two univalents. The multivalent configurations observed at diakinesis have also been traced at metaphase, either in profile or polar views. The observations on the former are much more difficult, owing to the large number of bivalents which overlap each other. Fig. 7 is a side-view metaphase showing a chain of three chromosomes and three univalents. A quadrivalent with sixteen other bivalents has been illustrated in polar view in Fig. 8.

The heterotypic anaphases are on the whole quite clean. The only minor irregularities observed at this stage were those arising from the anomalous behaviour of the univalents, wherever they occurred. The univalents seemed to divide after the members of the bivalents had disjoined to the opposite poles (Fig. 9). Although their divided halves were mostly included in the daughter nuclei, they were sometimes left in the cytoplasm (Fig. 10), where they degenerated. But for these minor irregularities, the whole meiotic process in the species is quite regular, resulting in the formation of normal tetrads. The pollen of the species is quite good, and its fertility very high.

The chromosome behaviour at the reduction division of an allotetraploid is to a certain extent correlated with the mode of chromosome pairing in the original diploid hybrid, by the doubling of which the amphidiploid arises. If there is non-conjunction or weak affinity between the different genomes of the parent species, the meiotic process in the allotetraploid will not be much disturbed by multivalent formation or interspecific pairing. Typical examples of this kind are *Raphano-brassica*

(Karpechenko, 1928), *Nicotiana digluta* (Clausen & Goodspeed, 1925), and *Aegilotriticum* (Tschermak & Bleier, 1926). However, several cases are known where the chromosome conjugation in the primary hybrid is good, and yet the meiosis in the allotetraploid is regular. Such is the case in the amphidiploid derivatives of *Solanum* (Jørgensen, 1928), *Saxifraga* (Whyte, 1930) and *Galeopsis* (Müntzing, 1932). Absence of quadrivalents in such cases is conditioned by two circumstances: (i) prevailing of autosyndesis over allosyndesis, and (ii) mechanical causes, viz. low frequency of chiasmata between homologous sets of chromosomes (Müntzing, 1932).

The fertility in allopolyploids is very much influenced by the course of meiotic division. Newly originated amphidiploids will show greater meiotic irregularities than the older ones, and would consequently be less fertile. Kihara (1931) finds that his newly arisen *Aegilotriticum* forms are less fertile than the relatively old strains of Tschermak. A similar tendency to stabilization in later generations has also been noticed in *Saxifraga* (Whyte, 1930) and in the *Nicotiana rustica-paniculata* (Lammerts, 1931) amphidiploids.

The time of origin of *Brassica juncea* is not known, but judging from the fact that its meiotic behaviour is very regular and its fertility high, it may be regarded as a fairly old species.

(ii) *Brassica campestris* L. var. *sarson* Prain ($2n=20$). The cytology of this species has been previously worked out by Alam (1936) and my observations confirm those made by him, excepting secondary association of chromosomes, which was not studied by me. At diakinesis and metaphase ten bivalents corresponding to the twenty somatic chromosomes were counted (Figs. 11, 12 respectively). No cases of plates with univalents were seen in the many cells examined, nor were any quadrivalents seen at diakinesis or other stages. The whole meiotic process in the species is regular and its pollen is good.

B. Cytology of the F_1 hybrid, *Brassica juncea* \times *B. campestris* var. *sarson*.

Owing to poor fixation of chromosomes in the root-tips of this hybrid, its somatic number could not be ascertained very correctly, but always about twenty-eight chromosomes, equal to the sum of the gametic number of the two parents, could be roughly counted.

At diakinesis about fifty nuclei were analysed and eighteen chromosomal bodies, consisting of ten bivalents and eight univalents, were always clearly counted (Fig. 13). At the earlier stage of diakinesis the univalents offer a sharp contrast to the bivalents, both in shape and size; but later, due to shortening and thickening of the chromosomes,

they attain a compact round form, and the distinction between the bivalents and univalents becomes less noticeable. The difference in the size of the various bivalents, corresponding to that observed in the somatic chromosomes of the two parental species, was marked.

Here the question arises; are the ten bivalents, which are consistently observed at diakinesis, formed by the autosyndetic pairing of the chromosomes of the two parents, or by the allosyndetic conjugation of the chromosomes contributed by each of them? The fact that F_1 hybrid *B. juncea* \times *Raphanus sativus* studied by Fukushima (1929) produces no bivalent chromosomes in its microsporogenesis shows clearly that the *juncea* chromosomes do not undergo synapsis *inter se* in the F_1 microsporocyte, when a dissimilar genome only is contributed by the other species. There is, therefore, strong reason to believe that ten chromosomes of *B. campestris* are homologous with ten chromosomes of *B. juncea*, and that these regularly pair to form the bivalents observed at diakinesis.

At metaphase I, out of about eighty cells counted, all showed eighteen bodies (Fig. 14) ($10_{II} + 8_I$) in polar view, excepting one cell which showed only sixteen (Fig. 15). The latter probably consisted of $1_{IV} + 9_{II} + 6_I$. The formation of a quadrivalent in the hybrid is according to expectation if we consider the fact that the two genomes (of ten and eight chromosomes each) of which *B. juncea* is composed, are of one common origin. This point has already been discussed when describing the meiosis of *B. juncea*.

In polar view the bivalents, which are round in shape, were seen to take up a regular position in the equatorial plane, whereas bar-shaped univalents generally lay on the periphery of the plate (Fig. 14). In profile the distinction between bivalents and univalents was apparent; the former lined up on the equator mid-way between the two poles, the latter generally scattered on the spindle (Fig. 16). Occasionally a few univalents were observed to lie on the equator along with the bivalent chromosomes. In Fig. 17 four such univalents are shown.

At anaphase I the members of the bivalents begin to disjoin first (Figs. 18, 19), while the univalents maintain the position previously held by them. After the disjoined halves of the bivalents have migrated to the poles the equatorial split in the univalents becomes apparent, but their actual division depends on the position held by them on the spindle. Those univalents which happen to be located near the equatorial plane line up at the equator, and their constituent chromatids disjoin to the opposite poles (Fig. 20). Sometimes both the halves were observed to move to the same pole (Fig. 21). The univalents which lie away from the

equator travel to the nearest pole undivided. Though, occasionally, all the univalents are included in the reforming daughter nuclei (Fig. 25), a few univalents or only halves of them, may lag on the spindle (Fig. 26) owing to their inability to reach the poles before the formation of the nuclear membrane. Richardson (1936) ascribes this slow movement of the univalents on the spindle to a weaker centromere charge. Each of the lagging univalents that is thus left stranded between the two interkinesis nuclei may in some cases form a separate small spindle, and the two chromatids constituting it pass to opposite poles. Chromatids with such a history are still traceable during the second division well out in the cytoplasm away from the remainder of the chromosomes (Fig. 23).

In cases where there is a sufficiently continuous series of lagging univalents from one telophase group to the other a restitution nucleus may be formed embracing the whole of the chromosomes. A metaphase II plate, with about twenty-eight chromosomes, is shown in Fig. 22, which is the result of a division I restitution nucleus. Formation of a restitution nucleus in this way will give rise to gametes having the diploid number of chromosomes, or approximately so. Any deviation from diploidy must be traced to random distribution of chromatids formed from univalents that divided at division I.

Formation of a restitution nucleus was first observed by Rosenberg (1927) in *Hieracium*. Since then this phenomenon has been found to be of frequent occurrence, both in animals and plants, including pure species, interspecific and intergeneric hybrids; but as regards the cause of restitution nucleus formation nothing definite is yet known. Rosenberg was of opinion that in *Hieracium* they were simply caused by the semi-heterotypic division, which brings about an uneven distribution of the univalents over the whole spindle, so that at anaphase-telophase a nuclear membrane is formed round all the chromosomes. Darlington (1930) found restitution nuclei in certain *Prunus* species and suggested that they may be caused by lagging of univalents at division I or divided univalents at division II. Sapehin (1933), however, working on wheat, suggests that they are not caused by univalence in meiosis, but are the result of the operation of Mendelian factors. In the hybrids of *Triticum durum* \times *T. vulgare* showing seven univalents at meiosis there were no diploid gametes formed, but such gametes were formed in F_4 plants with fourteen bivalents only. That the formation of a restitution nucleus is not always caused by univalence is shown by experience in other plants also. In the hybrid between *Nicotiana glutinosa* \times *N. tabacum* (Müntzing, 1935), at metaphase I most of the thirty-six chromosomes appeared as

univalents, and yet not a single dyad was observed. Lammerts (1929), on the other hand, found in the backcross progenies of *N. paniculata* \times *rustica* hybrid, where there was regular pairing according to the *Drosera* scheme, 32 % of viable gametes with the diploid number of chromosomes. Again, cases are known where comparatively less univalence has given rise to restitution nuclei. Mather (1937) has demonstrated cytologically the formation of unreduced gametes in *Prunus cerasifolia* with seven bivalents and two univalents. Gustafsson (1935), in *Hieracium*, found that the phenomenon of formation of restitution nuclei, though associated sometimes with univalence, was not caused by it. He found a marked difference between the constancy and appearance of the restitution nuclei in male and female organs in *Taraxacum*; and furthermore, while the restitution nuclei in the megaspores included all the zygotic number of chromosomes, many of the male nuclei were found with many chromosomes outside it. He also found that the number of such nuclei formed did not always correspond to the degree of univalence in different plants. Some apomicts with no trace of bivalents gave fewer dyads than those with many bivalents. It is, therefore, clear from the foregoing that univalence by itself is not a sufficient explanation for the formation of restitution nuclei. It is possible that some other causes, genetic and physiological, which are little understood at present, may also play a part in their production.

Täckholm (1922) has classified the irregularities in chromosome behaviour in heterotypic division of hybrids with unequal parental chromosome numbers into three groups, according to the affinity of chromosomes: (1) *Drosera* scheme with strong affinity, (2) *Hieracium-boreale* scheme with weak affinity, and (3) *Pygaera* scheme with no affinity. He again subdivided each scheme into three categories, by the behaviour of univalent chromosomes: (a) all univalents intact are distributed by chance to the poles, (b) some are distributed as a whole, while the others are split in the equator, (c) all univalents are split in the equator and their daughter halves are distributed to opposite poles, thus making nine types in all. The present hybrid, according to Täckholm's classification, therefore, belongs to category (1b), which he called *Pilosella* type. This type of behaviour of univalents was first described by Rosenberg (1917) in *Hieracium* subgenus *Pilosella*, and later in *Rosa centifolia major*, and *R. nutkana* \times *R. pendula*. Later studies have shown the same type of univalent behaviour in a large number of cases, such as *Nicotiana glabra* \times *N. glutinosa* (Clausen, 1927), *Triticum vulgare* \times *Aegilops* (Kagawa, 1928), triploid hybrid of *Raphanus sativus* \times *Brassica*

oleracea (Karpechenko, 1928) and various other *Brassica* hybrids (Morinaga, 1929; U, 1935).

Owing to anomalous behaviour of the univalents in division I, varying numbers of chromosomes come to lie at the metaphase plate during division II (Figs. 23, 24). Table III shows the frequency distribution of chromosomes in seventy-six metaphase II plates.

TABLE III

Frequency	No. of chromosomes on the homotypic spindle						
	13	14	15	16	17	18	Total
	2	2	32	18	18	4	76

At anaphase II one to three chromosomes were observed to lag on the spindle in different nuclei (Figs. 27, 29). These laggards must be those parts of the univalents which had divided during the first division, though, whether these lagging chromatids undergo a second splitting cannot be stated with certainty. Some of these were no doubt left out of the telophasic nuclei and were traceable in the cytoplasm at telophase II (Fig. 30).

Another interesting feature observed at this stage in the hybrid was the formation of a chromatin bridge (Fig. 28). By the side of the bridge a small chromatid is also seen. Whether this chromatid is a fragment, resulting from bridge formation, or a lagging half of a univalent, cannot be made out.

The formation of such a bridge in the hybrid indicates that one of the parents is heterozygous for an inversion in a single chromosome. When the chromosome with inverted segment pairs in the hybrid with its corresponding mate contributed by the other parent, a bridge can be formed if a cross-over occurs in the inverted segment.

As described elsewhere in this paper, two cross-overs are necessary for the formation of a second division bridge: one in the inverted region, and the other proximal to it in disparate relationship. In view of this hypothesis, it is a most surprising feature that in the present hybrid a second division bridge was observed, while no such bridge was observed at division I, although the latter can result by only a single cross-over in the inverted segment and must, therefore, be much more frequent than the former. The very rare formation of the bridge may perhaps be explained by the fact that the length of the inverted segment is so small as not to permit frequent crossing-over in that region. In any case the formation of a second division bridge is a sure indication of a proximal homologous segment beyond the inverted region.

Several cases are known in both plants and animals, where the presence of relatively inverted segments of chromosomes in pure species was revealed during a study of the interspecific hybrids between them. As instances may be quoted *Crepis divaricata* \times *C. Dioscoridis* (Müntzing, 1934), *Triticum dicoccum* \times *T. monococcum* (Mather, 1935), *Drosophila melanogaster* \times *D. simulans* (Pätau, 1935), *Anas platyrhynchos* \times *Cairina moscata* (Crew & Koller, 1936), *Lilium Martagon* \times *L. Hansonii* (Richardson, 1936), *Pisum humile* \times *P. arvense* (Håkansson, 1936).

Evidently due to very irregular meiosis in the present hybrid, gametes with varying numbers of chromosomes would be produced. It is, therefore, important to consider how far these gametes would be viable. It is difficult to come to any definite conclusion in this connexion till the F_2 or backcross progeny of the hybrid has been subjected to detailed study, but the results of some other workers are suggestive. Karpechenko (1927), from the determination of chromosome numbers in the F_2 of the cross *Raphanus* \times *Brassica oleracea*, as well as in triploid progeny derived therefrom by backcrossing, came to the conclusion that hyper- and hypodiploid gametes are non-functional. His observations led him to the view that "Besides the presence of definite chromosomes in the gametes for their viability is yet indispensable a definite equilibrium in their chromosome set." Richharia (1937a), however, observed in his *Raphanus-Brassica* hybrids that hypodiploid gametes did function. He attributes the non-viability of hypodiploid gametes in Karpechenko's material to lack of some genetical material, which was necessary for making them functional, or to some special effects of environmental conditions. Catcheside (1934) found in F_2 progeny of the hybrid *Brassica napus* ($n=18$) \times *B. rapa* ($n=10$) that plants with twenty-two to forty-five chromosomes were produced, showing thereby the viability of gametes over a very wide range. He observed, however, four modal points in the progeny: (1) at 27-29, that is, the neighbourhood of 28 in F_1 hybrid; (2) at 31, an inexplicable result; (3) at 34-35, that is, about six (the basic number of the swede according to his finding) more than the number in the parent, which perhaps means that these zygotes are better balanced than certain others; (4) at 42 (relatively triploid) which is half as many again as the parent, and means that diploid (unreduced) gametes had been functioning. He also found that about 13 % of the F_2 progeny was triploid, indicating a corresponding frequency of activity on the part of unreduced gametes. But since dyads in his material accounted for only 5-8 % of tetrads, it may be concluded that unreduced gametes were twice as viable, or active, as were subnormal reduced ones.

Also one plant in the F_2 which Catcheside thought to be an amphidiploid was markedly more fertile than the rest.

Although gametes with a wide range of chromosomes may be functional, yet not all the zygotes formed are viable. A certain percentage of ungerminated seeds would eliminate certain types from the progeny. McClintock in *Zea mays* put forth the view that the limiting factor in viability of the zygotes is not the inability of the female gamete to function but rather the capacity of the chromosome complement within the embryo to participate in growth and development.

The factors responsible for the elimination of gametes and zygotes have been the subject of discussion by many authors. Watkins (1932) explains the absence of certain expected classes due to pollen-tube competition. He states that "the normal relation of pollen tube to style is $1x : 2x$. If the relation is 1 : more than 2, pollen-tube growth is usually normal, but may sometimes be reduced. If the relation is 1 : less than 2, growth is usually greatly reduced." Müntzing (1933) thinks that the cause of disturbed seed development is of quantitative nature. During seed development three different tissues are in intimate contact, viz. embryo, endosperm and the surrounding somatic tissue of the mother plant. In normal cases the relation between these tissues is $2 : 3 : 2$. If the uniting gametes had different chromosome numbers, this relation would be altered, which would result in poor seed development, or complete abortion of seeds. Müntzing further thinks that if viable gametes with different chromosome numbers are formed by the same plant, and if those gametes form zygotes and seeds, the best seeds result from the union of gametes with the same chromosome number. But this explanation has not always been found to hold good, as perfect seeds are obtained in *Oenothera* (Capinpin, 1933), *Pyrus* (Darlington & Moffett, 1930; Derman, 1936) and *Allium* (Levan, 1935, 1936) by union of gametes with unequal chromosome numbers.

According to Winkler's view, viability is related to unbalance in the number of chromosomes. He considers that hyperdiploid forms are likely to differ more than tetraploids from the normal forms, owing to their internal unbalance in chromosomes. Bridges (1923), on the other hand, thinks that the effect of extra chromosomes on development depends on their internal gene balance. He found in *Drosophila* that triplo-X, or triplo-IV or haplo-IV flies were viable, but that no triplo-II or triplo-III appeared in the progeny of triploids. Since the numerical chromosome unbalance was the same in triplo-IV and triplo-III it appeared that the size of the extra chromosome had to be taken into account. Bridges'

view is further supported by work of East (1933) in *Nicotiana*. Among the progeny of triploids, he found plants having the same chromosome number showing variable fertility. This indicates that certain extra chromosomes fit more satisfactorily into general constitution than others. East's view, therefore, approaches that of Karpechenko, which has been outlined above.

The present hybrid was found to be totally self-sterile, but produced a few seeds by open pollination. The unfavourable weather conditions under which the hybrid was growing may also be responsible for its very low fertility, besides other factors. Under more favourable conditions, however, some new amphidiploid forms with higher chromosome numbers may be expected to arise in the F_2 progeny by the functioning of unreduced diploid gametes, the production of which in the meiosis of the F_1 hybrid has been demonstrated in the foregoing description. Some of these new amphidiploid forms may prove to be of greater economic value than those existing at the present time. The work in this connexion is, therefore, being continued.

The present account of the cross *Brassica juncea* \times *B. campestris* proves that *B. juncea* is composed of two genomes of ten and eight chromosomes, respectively, the first genome being derived from *B. campestris* (or some other twenty-chromosomal species). Morinaga (1934) has shown that the other genome of eight chromosomes is derived from *B. nigra* ($2n=16$), as the F_1 of the cross *B. juncea* \times *B. nigra* invariably showed $8_{II}+10_I$ at metaphase I. Therefore *B. juncea* is an amphidiploid derivative of the cross *B. campestris* \times *B. nigra*.

(ii) *Brassica Tournefortii* Gouan ($2n=20$) \times *B. trilobularis* H.f.T.
($2n=20$)

Twenty-five buds of *B. Tournefortii* were pollinated with pollen of *B. trilobularis* and eight pods were formed. Most of the pods, however, turned out to be empty; only two pods yielded seven seeds, which were sown for the present study. Although all the seeds germinated, only two seedlings turned out to be true hybrids. The other five plants proved to be apomicts, resembling the female parent in all morphological traits. U (1935) has also reported similar apomictic plants in the *Brassica* hybrids raised by him. The mode of origin of these apomicts is not known.

Table IV gives the comparative characters of the parental species and the F_1 hybrid between them.

A. *Cytology of the parents.*

(i) *Brassica Tournefortii* Gouan ($2n=20$). The somatic chromosomes of this species will be dealt with in Part II. Here only its meiosis will be described.

Diakinesis shows ten bivalents well spaced in the whole nucleus (Fig. 31). There is a well-marked difference in the size of the bivalents, five being bigger than the others. One bivalent corresponding to a pair of SAT-chromosomes in the somatic complement is seen in contact with the single nucleolus observed at this stage (Fig. 31). The number of chiasmata per bivalent ranges from one to three, some of them being still interstitial. In a few nuclei nine bivalents and two univalents were seen (Fig. 32). No quadrivalents or other multivalent configurations were observed, although a large number of nuclei were examined.

TABLE IV

	<i>B. Tournefortii</i>	<i>B. trilocularis</i>	F_1 hybrid
Leaves	Pubescent with pinkish midribs	Glabrous with green midribs	Pubescent with pinkish midribs
Flowers	Small, very pale yellow. Do not open fully	Larger in size, yellow. Open fully	Intermediate in size, yellow. Do not open fully
Anthers	Introrse	Introrse	Introrse
Pods	Short, compressed, sessile	Larger, flat and terete	(No pods obtained)
Seeds	Brown	Yellow	(No seeds obtained)

Fig. 33 is a polar view metaphase I with ten bivalents. The variation in the size of the bivalents at metaphase is less marked than at diakinesis. They can be roughly classified into five big and five small, which perhaps shows the allotetraploid nature of the species. The bivalents disjoin synchronously at anaphase, resulting in the distribution of ten chromosomes to each pole (Fig. 34). The second divisions are also quite regular, resulting in formation of normal tetrads. The pollen of the species is quite good.

(ii) *Brassica trilocularis* ($2n=20$). The cytology of this species has already been described by Alam (1936). The formation of ten bivalents both at diakinesis and metaphase observed during the present study (Figs. 35, 36) confirm the observations of Alam in this respect. One bivalent is seen in contact with the nucleolus at diakinesis (Fig. 35). This confirms the observation of one pair of SAT-chromosomes in the somatic complement (described later). The whole meiotic process of this species is quite normal.

B. *Cytology of the F₁ hybrid.*

In the root-tip cells of the *F₁* hybrid twenty chromosomes were counted (Fig. 125).

In the meiotic study of the hybrid the pairing of chromosome threads at stages earlier than diakinesis could not be analysed closely, due to their extreme slenderness. The observations had, therefore, to be confined to stages from diakinesis onwards.

At diakinesis the hybrid showed a great variation in the pairing of chromosomes in different nuclei—cases with total failure of pairing to those where a quadrivalent besides a varying number of bivalents had been formed, were observed. These have been sketched in Figs. 37–42. Never more than three bivalents were observed in any nucleus. Table V gives the frequency of pairing in forty-five nuclei, which were fully analysed at this stage.

TABLE V

No. of chromosomal bodies in the nucleus	Constitution of the chromosomal bodies	No. of cases
14	1 _{IV} + 3 _{II} + 10 _I	1
15	1 _{III} + 3 _{II} + 11 _I	2
16	1 _{III} + 2 _{II} + 13 _I	3
17	1 _{IV} + 16 _I	1
	1 _{III} + 1 _{II} + 15 _I	5
	3 _{II} + 14 _I	7
18	1 _{III} + 17 _I	4
	2 _{II} + 16 _I	6
19	1 _{II} + 18 _I	6
20	20 _I	10
	Total	45

The taxonomical classification of *Brassica* is as yet very imperfectly made out, but judging from the chromosome numbers of *B. Tournefortii* and *B. trilocularis*, they would probably be placed in the same section. Accordingly it would be expected that a hybrid between them will show a fairly high degree of conjugation of chromosomes, if not complete pairing, just as has actually been found in hybrids of other twenty-chromosomal species of *Brassica* (Morinaga, 1928; Richharia, 1937*b*; and also one hybrid described elsewhere in this paper). But this expectation has not been realized in the present hybrid. The very low degree of pairing of chromosomes in this hybrid may be accounted for by presuming that the chromosomes of the parental species have become structurally dissimilar during the course of evolution, which would bring about low frequency of chiasma formation and ultimate non-conjugation. This view

seems very likely when we consider the fact that the bivalents formed in different nuclei are invariably rod-shaped, having only one terminal chiasma, which indicates feeble homology. A similar feeble homology of chromosomes was inferred by Aase (1930) from the open type of pairing in *Aegilops*-rye and also in certain *Triticum* hybrids. Although the view outlined above seems quite likely, yet this conclusion has to be made with great caution, because, besides lack of homology, a whole series of genetic and environmental factors have been inferred by several workers to influence conjugation of chromosomes. For instance, in *Drosophila* (Gowen, 1928), *Zea* (Beadle, 1930), *Rice* (Ramanujam & Parthasarathy, 1935) and *Datura* (Bergner *et al.* 1934) the pairing of chromosomes has been determined to be controlled by specific genes. Peto (1934) attributes the irregular pairing in certain backcross segregates of the hybrid *Lolium* \times *Festuca*, to segregation of factors limiting chiasma formation. Sapehin (1933), working on wheat, came to the conclusion that failure of conjugation of chromosomes and other abnormalities of reduction division may be caused by the response of a particular genotype to the environment.

Again, external agencies like temperature have been demonstrated by several workers to affect conjugation of chromosomes (Katayama, 1931; Stow, 1926, 1927; Heilborn, 1930; Sax, 1931). Besides this, a number of cases are known where genetic homology of chromosomes alone is not sufficient for conjugation. Hollingshead (1930) found in a diploid *Crepis capillaris*, which arose by doubling of chromosomes in a haploid, gradations from full pairing to all chromosomes univalent. Meurman (1928) found in *Ribes* a marked variation in conjugation of chromosomes in different cells of the same anther, which he explains as being due to different nutritional conditions of the cells.

The occurrence of a ring of four in some nuclei in the present hybrid, which is a cross between species showing normal bivalent formation, may be explained on the assumption that the chromosomes of the two parental species do not correspond each to each, but have parts of their chromosomes relatively interchanged. Thus if one species has two pairs of chromosomes with the constitution $AB.AB, CD.CD$, and the other has chromosomes which may be represented as $AC.AC, BD.BD$, the hybrid between the two species will, following the assumption that only homologous parts of chromosomes pair, show a ring of four at meiosis, which may be represented as $AB.BD.DC.CA$. The formation of such a ring of four involves occurrence of four chiasmata, but if the length of two of the interchanged segments is not great enough to allow frequent

formation of chiasmata with homologous parts, the ring may then be replaced by a chain or several chains, as has been observed in *Oenothera* (Catchside, 1933) and rice (Parthasarathy, 1938). This would explain the high frequency of trivalents observed in the present hybrid.

Several cases are known in plants where hybrids with a ring of four have been obtained by crossing different homozygous races or species. As examples may be quoted, *Datura* (Blakeslee), *Pisum* (Håkansson, 1936; Sansome, 1929), *Polemonium* (Clausen), *Vicia* (Sveshnikova), *Triticum* (Smith, 1935) and *Allium* (Levan, 1936). Hybrids with a ring of six or two rings of four involving two interchanges have been produced either by crossing two parents which differed in two interchanges, as in *Datura*, or by crossing two single interchange races which have one gamete in common, as in *Oenothera* (Gates & Catchside, 1932), *Pisum* (Sansome, 1932) and in *Campanula* (Gairdner & Darlington, 1931).

Chromosome ring formation was first observed by Cleland (1922) in *Oenothera*, but Belling (1927) was the first to provide an explanation of ring formation on the hypothesis of segmental interchange. Burnham (1934) and McClintock (1931) have since provided the first concrete proof of the correctness of Belling's hypothesis, from a detailed study of chromosome pairing in a strain of maize known as "semi-sterile-2". On account of the peculiar morphology of its chromosomes, the two interchanged chromosomes could be distinguished on the basis of their length, position and size of the region of attachment. In prophase the pairing of homologous portions of such interchanged chromosomes gave rise to a cross-shaped configuration of four, which later opened out so as to give a ring of four at diakinesis by attachment of chromosomes only at their ends.

The existence of spontaneous forms which show ring formation at meiosis has also been demonstrated in a number of plant genera, such as *Campanula*, *Rhoeo*, *Humulus*, *Tradescantia*, *Anthoxanthum*, etc. Though Belling's hypothesis has given a clue to the behaviour of chromosomes in such forms, yet the exact mode of origin of these forms in nature is still very imperfectly understood. There is no direct evidence to show whether the interchange gave rise to the homozygote at once in nature, or whether it gave rise to new homozygous races which afterwards by crossing produced the heterozygote again. On the other hand, there is some reason to suppose from *Oenothera*, where homozygous forms are lethal and thus undergo elimination in nature, that such ring-forming types arose directly from the interchange without crossing between different parental zygotes (Darlington, 1937). In any case, the persistence in nature of permanent heterozygotes like *Oenothera*, and also

other ring-forming plants like *Campanula* and *Rhoeo*, which at the present moment appear to be tending towards permanent heterozygosity, would lead to two important conclusions: first, among the many structural changes that are characteristic of species evolution, segmental interchange occupies the prominent position, and secondly, segmental interchange has not only a survival value, but under certain conditions it has been specially favoured by nature.

Artificially, segmental interchange between non-homologous chromosomes has been produced by X-rays in *Drosophila* (Muller, 1930; Dobzhansky, 1931;), *Zea* (McClintock, 1931, 1933; Anderson, 1935), *Oenothera* (Catcheside, 1935), *Triticum* (Katayama, 1935), *Oryza* (Ramiah *et al.* 1934; Parthasarathy, 1938). Segmental interchange between homologous chromosomes is the result of "crossing-over", which is conditioned by their pairing due to primary attraction during the earlier stages of meiosis, but no such pre-existing conditions are known to account for segmental interchange between non-homologous chromosomes.

The evidence on the pairing of non-homologous chromosomes or portions of them is very conflicting. McClintock (1933) and Burnham (1934) found in *Zea* an intimate association of non-homologous segments during the prophase, but no chiasmata were formed in this region, and consequently this association was not seen to persist to metaphase. On the other hand, Gates (1908) found in a triploid *Oenothera* ten and eleven chromosomes regularly moving to the poles at anaphase I. Yarnell in *Fragaria* (1931) and Longley in *Rubus* (1924) and *Citrus* (1926) also inferred a certain frequency of non-homologous pairing of chromosomes. More recently, Lammerts (1934), working on haploid *Nicotiana*, not only finds non-homologous parts paired at pachytene, but adduces evidence in favour of the view that such pairing can, and often does, persist until metaphase, by the formation of chiasmata between paired non-homologues.

The phenomenon of reciprocal translocation involves a mechanism by which chromosomes break at random and the broken ends subsequently get fused. Morgan & Sturtevant (1925) have suggested that translocations may be caused by interlocking of non-homologous chromosomes during synapsis. If the interlocking between bivalents is not released, any strain may bring about breakage of the chromatids at the point of contact, so that a section of one chromosome might become attached to another non-homologous chromosome. The interlocking of bivalents observed by Gelei (1921), Sax (1930) and Catcheside (1932) is of much significance in this connexion.

The evidence from irradiation experiments suggests that only broken ends of chromosomes can fuse. The fragments are never known to unite with whole ends of chromosomes. Stadler (1932) thinks that an unlimited time interval, extending even to several cell generations, may lapse between the breakage of chromosomes and the reunion of broken ends. But the evidence from the behaviour of acentric fragments at mitosis does not support his view. Catcheside (1935), on the other hand, thinks that when breaks occur at the point of overlap of two chromosomes, the broken ends immediately rejoin at random. This view seems very reasonable.

All the configurations observed at diakinesis in the present hybrid were clearly made out in side view of metaphase I also. These have been illustrated in Figs. 42-49. The metaphases, on the whole, presented a very irregular appearance; while all the bivalents and multivalents lined up on the equator, the univalents were irregularly scattered over the whole spindle or were located at both the poles (Figs. 44-47). A varying number of univalents was sometimes observed to lie on the equator along with other paired configurations. Occasionally most of the univalents were seen to form a perfect equatorial plate. In Figs. 48 and 49, which are side view metaphases, ten and eight univalents, respectively, are lying on the equator. In Fig. 43, which is a polar-view metaphase, excepting two univalents, all the rest are located in the equatorial plane.

The orientation of multivalent bodies, consisting of more than two spindle-attachment regions, on a bipolar spindle is bound to be irregular. The disjunctional arrangement of such bodies on the metaphase plate is bound up with the question, whether the multivalent is physically capable of lying in one axis, as does a bivalent. In Fig. 49 the ring of four is orientated on the plate in such a manner that each of its four centromeres is located on an independent axis. With such an arrangement the chromosomes making up the ring may divide into two and two or into three and one, depending upon the way in which forces responsible for the anaphasic separation of chromosomes act. In Fig. 61 a group of three chromosomes is seen passing to the upper pole. Possibly this group has resulted from the division of a ring of four chromosomes into three and one. Similarly, the trivalents represented in Fig. 46-48 are arranged on the plate in a linear order. These may divide into three whole chromosomes or into two and one. The latter condition is actually seen in Fig. 48. Such irregular division of multivalents is liable to cause uneven distribution of chromosomes to the resulting daughter nuclei.

At anaphase I the components of the paired chromosomes were the first to disjoin to the opposite poles. The division of univalents was determined by the position held by them on the spindle. Those univalents, which happened to lie on the equator along with the paired chromosomes seemed always to undergo division, while the other univalents passed intact to the nearest pole. The divided halves of the univalents may migrate to opposite poles or to the same pole. The latter condition is shown in Fig. 55. In a majority of cases the univalents, whether divided or undivided, were included in the daughter nuclei, but a few of these occasionally lagged on the spindle, and were traceable at division II in the cytoplasm away from the other chromosomes (Fig. 58). Very frequently the lagging univalents bridged the two poles and a single restitution nucleus resulted (Fig. 63). The restitution nucleus may enclose all the chromosomes, or a few may occasionally be left out, as in Fig. 63. The production of gametes with diploid, hyper- or hypodiploid number of chromosomes is thus governed by the constitution of the restitution nucleus. In Fig. 50 a second division metaphase plate with twenty-two chromosomes, which is evidently the result of a division I restitution nucleus, has been illustrated. The causes leading to the formation of a restitution nucleus have already been discussed in the hybrid *Brassica juncea* \times *B. campestris*.

Fig. 62 illustrates a univalent bridge observed at first anaphase. The formation of such a bridge indicates the presence of an inverted segment in a single chromosome. Univalent bridges arise from lagging members of trivalents (or univalents) which have formed a chiasma in the inversion and one proximal to it, with one of its partners, and since it is part of a trivalent, it must have formed at least one chiasma with the other partner. During the post-pachytene stages up to anaphase one of its arms forms a closed loop. At anaphase its centromere, which is lagging on the equator, divides precociously and the arm which was in the form of a loop stretches out to form a bridge. Thus the conditions leading to the formation of a univalent bridge are evidently the same as those which would give a second division bridge by the chromatids of a bivalent. In order that a univalent bridge shall be formed, the chromosome which has crossed over and formed chiasmata with its partners must be left on the equatorial plate at anaphase. This depends largely upon the way in which trivalents are orientated at metaphase, which in turn is governed by the number and position of their chiasmata. According to Darlington (1937) the lagging members largely arise when the orientation of a trivalent is "linear" or "indifferent". In other plants univalent bridges

have been recorded in *Tulipa* (Upcott, 1937), *Allium* (Mensinkai, 1939) and *Calocolaria* (Srinath, 1939). In *Brassica* such a bridge has not been reported before.

At anaphase II those univalents which had not divided in the first division divide equationally and their split halves pass to opposite poles, while the univalents which had divided in the first division lag on the spindle (Figs. 65, 67). Some of these laggards were observed to undergo a second splitting (Figs. 65, 66) and the products of their division passed either to opposite poles or the same pole. Morinaga (1929*a, b*) observed a similar double division of the univalents in F_1 crosses of *B. napella* with various ten-chromosomal species. In certain cases a regular double division of univalents has resulted in the production of viable gametes with diploid number of chromosomes which, by backcrossing the F_1 hybrid to either of the parents, gave rise to triploid progeny. In the cross *Pygaera curtula* ($n=29$) \times *P. anachoreta* ($n=30$) Federley observed total absence of conjugation of chromosomes and double division of the univalents at meiosis to give diploid gametes. When this hybrid was backcrossed to *anachoreta*, nearly all the progeny were triploid with $2n=89$ chromosomes. In plants, Ramanujam (1937*a*) was the first to give an experimental demonstration of the origin of polyploidy through formation of diploid gametes by double division of univalent chromosomes.

It is interesting to consider how such a regular double division should take place in univalents in some cases, while it is the general rule that they divide only once. In *Hyacinthus* (Naithani, 1937), hybrid ducks (Crew & Koller, 1936) and *Zea mays* (Beadle, 1931), supernumerary divisions are known to occur at meiosis, which are supposed to be genetically determined. Meurman (1928) found in *Ribes* that double division of chromosomes was characteristic of certain cells which showed no pairing of chromosomes, while other cells with more or less bivalents did not show this peculiarity. Darlington (1930) points out that lack of pairing among chromosomes is apparently a condition equality of the suppression of first division and of double division of chromosomes. Lack of pairing may be a necessary condition, but that it is not the cause of double division of the chromosomes is shown by the fact that many plants with practically no pairing fail to show this phenomenon. Our state of knowledge at the present time does not fully explain the cause of occurrence of double division as a means of diploid gamete formation.

Some other interesting features observed during second division in the present hybrid are:

- (i) *Formation of supernumerary spindles.* Frequently more than two

homotypic spindles were observed (Fig. 64). These supernumerary spindles arise from univalents which were left in the cytoplasm as a result of lagging in division I. This process leads to the formation of micronuclei (Fig. 71). Morinaga (1929) and Catcheside (1934) observed a similar phenomenon in the microsporogenesis of the *Brassica* hybrids studied by them.

(ii) *Fusion of homotypic spindles.* The homotypic spindles in the present hybrid were formed perpendicularly, obliquely or parallel to each other. When parallel spindles are formed very closely they may fuse to give rise to two anaphasic groups instead of four (Fig. 68). This would naturally result in the formation of dyads instead of tetrads, which will ultimately give rise to diploid gametes. The fusion of homotypic spindles has been observed in several other plants, e.g. *Raphanobrassica* (Karpchenko, 1928), *Galeopsis* (Müntzing, 1930). The polyploid progeny obtained in the first two cases is attributed by the authors to the probable formation of diploid gametes by this process.

(iii) *Union of two daughter chromosome groups.* At anaphase II, occasionally the two anaphasic groups of a spindle formed a restitution nucleus (Fig. 69). This leads to the formation of triads in place of tetrads.

The hybrid showed dyads, monads, triads to the extent of 25, 8 and 5% respectively. In spite of the fact that unreduced gametes were so commonly produced, the hybrid turned out to be totally sterile. This may largely be attributed to extremely unfavourable weather conditions under which it was growing. All attempts to backcross it with either parent also failed, when the hybrid was used as a female. Backcrossing could not be done the other way as the anthers of the hybrid were never seen to dehisce and shed any pollen.

(iii) *Brassica trilocularis* H.f.T. ($2n=20$) \times *B. rapa* L. ($2n=20$).

Of the forty buds of *B. trilocularis* pollinated with pollen of *B. rapa*, thirty-four set pods, which produced one hundred and seventy-eight seeds. Forty F_1 seeds were sown for cytological material, all of which germinated. The F_1 plants were very vigorous and grew to a much greater height than either of the parents. Table VI gives the comparative characters of the parental species and the F_1 hybrid between them. The cytology of *B. trilocularis* has been dealt with earlier in this paper.

B. rapa also shows ten bivalents both at diakinesis (Fig. 72) and metaphase (Fig. 73), one bivalent being attached to the nucleolus at the former stage. No multivalents or univalents were seen at any stage. The whole meiotic process in the species is quite normal.

In the hybrid *B. trilobularis* × *B. rapa* there is a complete conjugation of chromosomes resulting in the regular formation of ten bivalents at diakinesis (Fig. 74) and metaphase I (Fig. 75). At anaphase I three cells out of about sixty showed non-disjunction of a bivalent which resulted in the distribution of eleven and nine chromosomes to opposite poles (Fig. 76). Richharia (1937*b*) observed a similar high frequency of non-disjunction in the cross *B. pekinensis* × *B. rapa*. But for this irregularity, the whole meiotic process in the hybrid is quite normal, pollen sterility being less than 2%. The hybrid sets pods and seeds freely under open pollination.

TABLE VI

	<i>B. rapa</i>	<i>B. trilobularis</i>	<i>F</i> ₁ hybrid
Leaves	Pubescent	Glabrous	Pubescent
Flowers	Large, deep yellow	Yellow with narrow petals	Deep yellow; intermediate in size
Anthers	Extrorse	Introerse	Extrorse
Pods	Long, cylindrical	Short, flattened	Intermediate
Seeds	Brown	Yellow	Brown

A very interesting feature noticed in the present hybrid was the persistence of nucleolus to metaphase I in a very large number of cells. At later stages the persisting nucleolus seemed to fragment into a number of smaller bodies which were seen to lie in the cytoplasm until telophase II. Fig. 77 is a cell in anaphase I showing five fragmented nucleoli, while Fig. 78 shows the same condition at telophase II. Persistence of nucleolus in meiotic stages is very rare and has been reported in only a few cases.

PART II. CYTOLOGY OF *BRASSICA* SPECIES

A. Somatic chromosomes

Table VII shows the somatic chromosome numbers of ten species, along with the number of satellites and nucleoli and the frequency of various types of chromosomes present in each. Since the primary constrictions could not be seen clearly owing to the small size of the chromosomes, their classification has been based solely on the length. The interesting feature in this connexion is that the frequency of various types of chromosomes is exactly the same in all the twenty-chromosomal species examined. The sixteen- and eighteen-chromosomal species also have the same chromosome types, with the exception that in the former four and in the latter two chromosomes of the short type are non-existent. The significance of this difference will be dealt with later. In any case the existence of more or less similar chromosome types in these

species would justify the supposition of a common origin for all of them. This point will be elaborated from other evidence later on.

As shown in Table VII, there is in *Brassica* a complete correspondence between the number of satellites and nucleoli in the somatic complement. The relation of satellites to nucleoli has been dealt with at length in a paper on *Narcissus*. The numerical correspondence between the number of satellites and nucleoli holds good also in several other plant genera, e.g. *Zea* (McClintock, 1934), *Vicia* (Heitz, 1931), *Aloinae* (Sato, 1937), *Triticum* (Bhatia, 1938).

Another important point to consider from the data presented in Table VII is the relation of number of satellites and nucleoli with the phylogeny of various species. De Mol (1927) pointed out first in *Hyacinthus* that the number of nucleoli in telophase increases with polyploidy,

TABLE VII

Serial no.	Name of the species	Chromosome no. (2n)	No. of		Chromosome types					Ref. to figure
			Satellites	Nucleoli	Longest	Long	Medium	Small	Smallest	
1	<i>B. nigra</i>	16	4	4	2	8	6	—	—	79, 91
2	<i>B. oleracea</i>	18	2	2	2	8	6	2	—	80, 92
3	<i>B. rapa</i>	20	2	2	2	8	6	2	2	81, 93
4	<i>B. campestris</i>	20	2	2	2	8	6	2	2	82
5	<i>B. trilobularis</i>	20	2	2	2	8	6	2	2	83
6	<i>B. Tournefortii</i>	20	2	2	2	8	6	2	2	84
7	<i>B. monensis</i>	n=12	2*	—	—	—	—	—	—	—
8	<i>B. juncea</i>	36	6	6	4	16	12	4	4	85, 94
9	<i>B. rugosa</i>	38	—	4	—	—	—	—	—	86, 95
10	<i>B. napus</i>	38	—	4	—	—	—	—	—	87

* The number of satellites has been judged from attachment of one bivalent to nucleolus at diplotene.

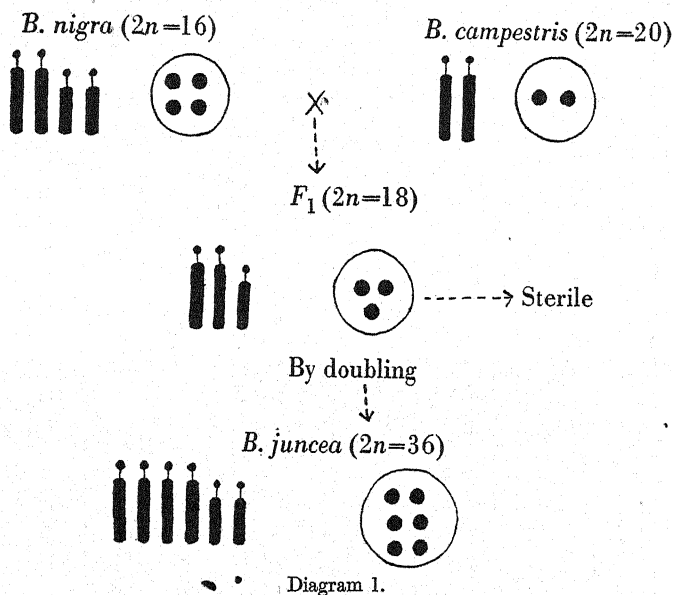
diploids having two nucleoli, triploids three, tetraploids four and so on. Each satellite and nucleolus in the somatic complement represents one haploid set of chromosomes in the species. This point, although very incompletely studied at present, has already found support in many plants. Gates (1937) has given a succinct review of the relevant literature. Nandi (1936) found two and four bivalents attached respectively to the nucleolus in secondary tetraploid and octaploid species of rice. Ramanujam (1937a) has shown the presence of two nucleoli in a diploid and three in an autotriploid rice. Bhatia (1938) has demonstrated the occurrence of four satellites and four nucleoli in a tetraploid wheat, and six satellites and six nucleoli in a hexaploid. Thus, if the principle that diploids have a single pair each of SAT-chromosomes and nucleoli is of general application, *Brassica nigra*, which has been shown to possess two pairs of SAT-chromosomes and four nucleoli, can be inferred to be a tetraploid. Judging from the somatic chromosomes, as well as the meiotic behaviour,

of this species (described later) it appears to be an allo- rather than an autotetraploid. The primary basic number of *Brassica*, as judged from the secondary association of chromosomes at meiosis (described later), appears to be five. On the basis of $b=5$ the derivation of the chromosome complement of *B. nigra* may be explained as follows: The original haploid set of five chromosomes may be represented by A . By gene mutations, or structural changes such as translocations, inversions, interchange or reduplication, another type having a different constitution, say A^1 , may arise. A cross between A and A^1 would be at least partially sterile due to low frequency of pairing. A concrete example of this situation is afforded by the cross *B. Tournefortii* ($2n=20$) \times *B. trilocularis* ($2n=20$), described later in this paper, where 0-3 bivalents are formed. Another example is *Nicotiana Bigelovii* \times *N. suaveolens* (Goodspeed & Clausen, 1927), which showed 0-1 bivalent only. Now by the occurrence of amphidiploidy in the cross $A \times A^1$ (cf. Karpechenko, 1927, 1928) the tetraploid number twenty would be obtained. Loss of four chromosomes from this number by somatic or meiotic irregularities would result in the production of the $2n=16$ type found in *Brassica nigra*. The absence of four small chromosomes in the idiogram of this species, as compared to that of twenty-chromosomal species (cf. Table VII) points almost with certainty towards such a loss having actually occurred in the evolution of the species. Manton (1932) has also inferred similar losses of chromosomes in several other genera of the order Cruciferae, e.g. Hesperidinae.

A still more noteworthy feature of Table VII is that whereas *B. nigra*, which has the lowest chromosome number known in the genus, possesses two pairs of satellites and four nucleoli, various eighteen-, twenty- and twenty-four-chromosomal species examined show only two satellites and two nucleoli. This fact, therefore, strongly suggests that the latter species must have lost the extra pair of satellites by mutation. That such a loss in polyploid species is possible and has actually occurred in many plant genera will be discussed later on. It seems quite logical to believe that the forms in which such a loss has occurred may be of more remote origin than those which have retained the original duplicated set of satellites. The comparison of the idiogram of *B. oleracea* ($2n=18$) with that of twenty-chromosomal species shows that it has arisen by the loss of the smallest pair of chromosomes.

The presence of six satellites and six nucleoli in *B. juncea* is to be explained by the fact that this species has arisen by amphidiploidy from a cross between *B. nigra* with four satellites and one of the twenty-chromosomal species with only two satellites. The F_1 of the cross between

these two species would have three satellites and three nucleoli, and being sterile due to chromosome unbalance, must double its number, if it were to persist. This would give rise to a species with $2n=36$ chromosomes which would have three pairs each of satellites and nucleoli. The results of interspecific hybridization (described earlier) have shown in an unmistakable way the amphidiploid nature of this species. The presence in it of six satellites and six nucleoli lends an extra proof in this direction. The origin of *B. juncea* represented as in Diagram 1.



Although *B. rugosa* and *B. napus* both have higher chromosome numbers than *B. juncea*, the number of nucleoli in each of them is less (cf. Table VIII). This situation can be similarly explained (Diagram 2). *B. napus* has already been synthesized by U (1935) from the cross *B. campestris* \times *B. oleracea*, which leaves no doubt about the amphidiploid origin of this species. However, the evidence from the number of nucleoli provides an extra proof in this direction.

No interspecific hybridization has so far been attempted with *B. rugosa*, and, therefore, the exact nature of this species is not known. But judging from the fact that it has the same chromosome number and also shows the same number of nucleoli as *B. napus*, it may be inferred that it is also an amphidiploid. Since its morphological characters are somewhat different from *B. napus* it may be concluded that either the species from

the cross of which *B. rugosa* arose are different from those which gave rise to *B. napus*, or the difference between the two species is the result of peculiar adaptation to the environmental condition under which each of them has been growing. In this connexion it may be mentioned that *B. rugosa* is at present in cultivation in Burma, while *B. napus* is a European species. It is difficult to come to any definite conclusion on this point till a more detailed study has been undertaken.

Regarding the mitotic process, the following interesting features require special mention.

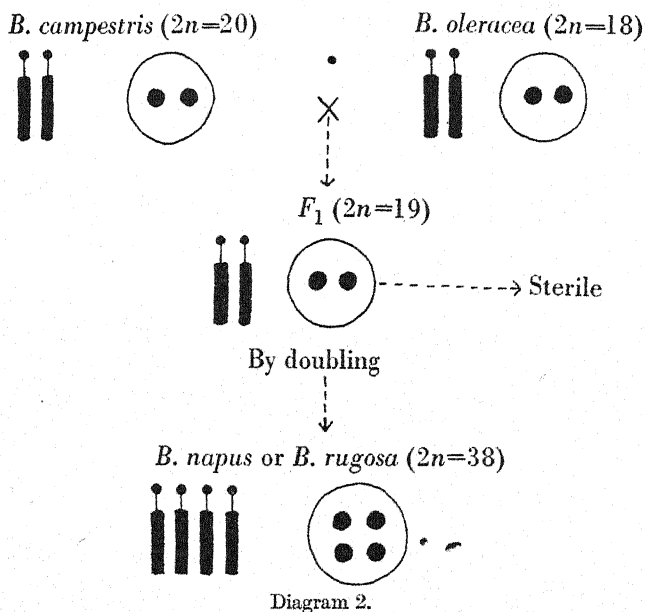


Diagram 2.

(i) *Somatic pairing.*

The paired arrangement of certain chromosomes at metaphase was a characteristic feature of nearly all the species examined. The degree of pairing varied considerably in different cells and in different species. It was more marked in species with low chromosome numbers (viz. $2n=16, 18$ and 20) than in species with higher numbers ($2n=36, 38$). Evidently, the higher number of chromosomes offers some mechanical difficulties in the way of homologous pairs coming within the sphere of attraction. The most extreme case of pairing was seen in one cell of *B. campestris*, where the chromosomes of more or less the same size and shape sorted themselves into separate groups which were located on the

periphery of the plate, thus leaving the interior of the spindle hollow. This is shown in Fig. 88, from which it is clear that almost all the longer chromosomes in the complement are located on one side of the plate and smaller ones on the other side. Again, the chromosomes of each type have formed smaller groups of two or more, the chromosomes in each group lying parallel or radially. This characteristic grouping of more than one pair of chromosomes is not only an indication of homology between the parental chromosomes, but seems also to be a manifestation of residual ancestral homology. This condition approaches very near to the secondary association of chromosomes at meiosis and is, therefore, suggestive of the polyploid nature of the species. Metz (1916) observed in certain tetraploid cells of *Diptera* that the chromosomes were associated in groups of four and eight at prophase rather than in pairs.

Darlington (1937) assumes that somatic pairing does not usually show itself at mitosis in plants with secondary pairing at meiosis. This idea is contrary to the observations of Skovsted (1933) and Davie (1933) in *Gossypium*, Parthasarathy (1938) in rice, and myself in *Brassica*, where there is secondary pairing in meiosis and somatic pairing at mitosis.

Somatic pairing in plants was first reported by Strasburger (1905). Since then it has been reported in many plant genera, such as *Oenothera* (Gates, 1912), *Galtonia* (Newton, 1924), *Sorghum* (Huskins & Smith, 1932), *Gossypium* (Skovsted, 1933, Davie, 1933), *Cicer* (Iyengar, 1939). In many species of *Diptera* somatic pairing is particularly marked (Metz, 1916).

(ii) *Variations in chromosome number.*

The somatic chromosome number of *Brassica Tournefortii* (see Table I) is twenty, but one cell with twenty-four chromosomes was come across (Fig. 89) in a root-tip showing the normal number of chromosomes in other cells. From the figure it is clear that the chromosomes in the cell are arranged into two more or less separate groups, one of sixteen chromosomes and the other of eight. Furthermore, the chromosomes in the latter group are seen to be distinctly arranged in four pairs, each pair being almost of the same size and shape. This characteristic paired condition of the chromosomes justifies the conclusion that the present increase in their number must have occurred by precocious splitting of four chromosomes, and separation of the daughter halves during prophase.

Gates (1912) observed in *Oenothera lutea* a variation of fifteen to twenty-one chromosomes in different cells of the nucellus and accounts for the higher numbers of chromosomes by separation of certain of the

daughter chromosomes immediately after their formation by the prophase split. He thinks, however, that the increased chromosome number may not be perpetuated, for the chromosomes which separated precociously may merely segregate in metaphase instead of dividing again, and thus restore the normal number.

Sudden alterations in chromosome number may also be brought about by several other causes, such as "lagging", "non-disjunction", and fragmentation. When the halves of a split chromosome in somatic tissue pass to the same pole instead of separating, hyperploid and hypoploid nuclei result. Such an aberration may have an immediate visible effect upon the tissue concerned, giving one type of "somatic mutation", but it affects the next generation only if it occurs in the lineage of the reproductive cells. Diploid *Daturas*, for example, have been seen to bear $2n+1$, $2n-1$ and $4n$ branches. In *Drosophila* a large number of sex mosaics (gynandromorphs) have arisen owing to somatic elimination in one of the early cleavage divisions of one member of the X-chromosome pair (Morgan & Bridges, 1919). Similarly, the so-called "diminished" mosaics have been shown by Bridges (1921) to be due to haploidy for the fourth chromosome. Mohr (1932) describes a "minute" mosaic which showed only one member of the fourth chromosome in the cells of the ovaries.

Sporadic aberrations in chromosome numbers during somatic mitosis have frequently been reported in a large number of plants by various workers. Farmer & Shove (1905) found the chromosome number to vary between twenty-six and thirty-three in different cells of *Tradescantia virginica*. Upcott (quoted by Darlington, 1937) observed the chromosome number to vary from four to nineteen in different nuclei of the same plant of *Tulipa galatica*. Darlington thinks that such wide variations can come about only where the chromosomes are inert, and therefore do not affect the growth of cells by changes in their number. Swezy (1937), in examining the progeny of a triploid plant of *Crepis Bungei*, found the chromosomes to vary between twelve and seventeen in different cells of the same individual. Occasionally, one whole region of an individual may differ from the remainder in chromosome number, e.g. in *Datura* (quoted by Sharp, 1934). Sometimes the modified region is a well-defined sector of the plant, e.g. in *Crepis* (Navashin, 1926, 1930; Hollingshead, 1930), *Lycopersicum* (Lesley, 1925); and *Nicotiana* (Ruttle, 1928).

(iii) *Chromatin bridges in somatic mitosis.*

In one cell of *Brassica trilocularis* the formation of a chromatin bridge along with a fragment was observed at anaphase (Fig. 90). The origin of such a condition may be explained on the assumption that a break occurred at the point of overlap of two chromosomes during the previous interphase and the broken ends subsequently fused. Since somatic pairing of certain chromosomes has been found to be a characteristic feature of *B. trilocularis*, as of other *Brassica* species, it is quite possible that the overlapping chromosomes may be one of the homologous pairs in the somatic complement. The present bridge is, therefore, similar in nature to those formed through break and reunion between chromosomes or chromatids as a result of X-ray treatment (Husted, 1936). Upcott (1937) observed the formation of similar kinds of bridges with fragments due to spontaneous chromosome changes during the first post-meiotic mitosis in pollen grains of *Tradescantia virginiana*. Barber (1938) has also reported the existence of chromatin bridges in pollen-grain divisions of *Paeonia Veitchii* and *Kniphofia rufa*. He did not, however, observe any fragments, and therefore assumed that these bridges originate by the union of homologous ends of two sister chromatids derived by division from one parental chromosome. Mensinkai (1939) observed a somatic bridge in root-tip of *Allium margaritaceum* and assumes that the formation of such bridges is due to some adverse external circumstances influencing the physiology of the particular nucleus, producing denaturation of chromatin in the particular chromosome, which results in the fusion of ends of sister chromatids before they separate at anaphase. Irradiation experiments have, however, shown that there is a fundamental difference between the whole and broken ends of chromosomes with regard to their fusibility. On these results, therefore, Barber's and Mensinkai's explanation for formation of somatic bridges does not seem to be adequate.

B. *Meiosis of Brassica species*(i) *Brassica monensis* Huds. ($n=12$).

This is a wild species found in western Europe and chiefly in the Pyrenees and south-western Alps, but extends to the west coast of France and Britain, where it is found as far north as Bute.

The somatic number could not be determined owing to the very small quantity of seed available, which was used exclusively to grow plants for meiotic study. Its chromosome number ($n=12$) has, therefore, been determined from meiotic stages only.

The nucleus of the pollen mother cell passes into synapsis with only one nucleolus. Owing to the very small size of the nucleus and slenderness of the threads, study of zygotene and early diplotene is practically impossible. The earliest stage well fixed for critical study was late diplotene. At this stage twelve bivalents can be counted in the nucleus (Fig. 96), although with difficulty, because, the chromosomes not having fully contracted, the bivalents overlap each other. In a large number of cells one bivalent was seen attached to the nucleolus (Fig. 96), which would mean the presence of one pair of satellites in the somatic complement. This fact, however, requires confirmation from the study of the somatic chromosomes. Bivalents of four shapes—V, X, O or 8—could be distinguished in different nuclei, the first two forms having a single chiasma each, the third two, and the fourth three chiasmata. These various forms of bivalents have been illustrated separately in Figs. 97*a-h*. The chiasmata in most of the bivalents are still interstitial at this stage, although in a few of them they are already fully terminalized.

With the finish of diplotene stage the bivalents contract markedly and the repulsion between them increases, so that at early diakinesis they are scattered throughout the nucleus. At the same time the chiasmata of each bivalent undergo still more terminal movement, so that bivalents of two forms—rings with two chiasmata each and rods with only one terminal chiasma—result. A few of the bivalents still retain interstitial chiasmata, which may persist to metaphase I. The persistence of these interstitial chiasmata can be more conclusively inferred from the lagging that is shown by certain bivalents at anaphase (Fig. 105).

At mid-diakinesis twelve bivalents in each nucleus could be clearly counted (Fig. 98), although very rarely nuclei with one ring of four chromosomes and ten bivalents were found (Fig. 99). The rare formation of a quadrivalent in this species, which is most probably an allotetraploid, as judged from the secondary association of chromosomes described later on, is most easily explicable if it be supposed that the duplicated chromosomes have become structurally dissimilar by linear rearrangement of their parts. Structurally altered chromosomes of the same ultimate origin will then be homologous continuously over short lengths, and therefore unable to pair regularly when in competition with unaltered chromosomes. Occasional pairing and chiasma formation between them would result in multivalents.

From mid-diakinesis onwards the chromosomes contract still further and the two members of a bivalent thicken and shorten, and also come closer to one another. At the same time bivalents move towards the

centre. This converging movement suddenly accelerates and results in the termination of diakinesis and the very close assemblage of bivalents in the centre of the nuclear area. The nuclear membrane and nucleolus disappear. Kuwada & Sugimoto (1928) inferred from the difference in the staining reactions between chromosomes confined within the nuclear membrane and those free in the cytoplasm, that they change from being electro-negative in the former case to electro-positive on the spindle. The "prometaphase" stage, therefore, may be the result of weakening of the electric charge which keeps the bivalents well spread at mid-diakinesis.

At metaphase I the twelve bivalents assume an arrangement similar to that of floating magnets in an electric field, the commonest arrangement being three inside and nine on the periphery, which is regarded as the stable form by several workers. Mayer (1879), Cannon (1923) and Kuwada (1929) have dealt fully with the theory governing the arrangement of chromosomes at metaphase, and it seems unnecessary here to go into the details of that problem. One noteworthy thing, however, is that at first metaphase a variable number of bivalents is secondarily paired, and this distorts to a large degree the arrangement of chromosomes expected on the theory of floating magnets. Alam (1936) in some other species of *Brassica*, and Iyengar (1939) in *Cicer*, came to the same conclusion, although Nandi (1936), in rice, observed that the secondarily paired bivalents behave as a unit and still retain the theoretical arrangement on the metaphase plate.

The later meiotic process in this species is mostly regular, resulting in the formation of tetrads with twelve chromosomes in each nucleus. A few aberrations, however, call for special attention.

(a) It has been noted above that pairing at diakinesis and metaphase I is conditioned by more or less terminal chiasmata; failure to form any chiasmata between the chromatids of the two associated chromosomes results in the formation of univalents. The presence of two such univalents at metaphase was observed in many cases, both in polar and side views (Figs. 103, 102). Univalents are perhaps most frequently formed by the smallest pair of chromosomes and by those with a subterminal attachment constriction. The presence of univalents need not necessarily mean deficient gametes, provided that at division I they go at random to opposite poles of the spindle, and are not omitted from the nuclei at interkinesis. Two univalents have a 50% chance of passing to opposite poles. However, from the counts of chromosomes made at metaphase II, three cells were come across which showed a distribution of thirteen and

ten (Fig. 104). In such cases one univalent has evidently been lost in the cytoplasm during the first division.

(b) Another interesting feature noted was the lagging of a bivalent on the spindle at metaphase I (Fig. 105). This is most probably conditioned by the persistence of interstitial chiasmata to metaphase. Catcheside (1934) observed a similar phenomenon in *Brassica napus* ($2n=36$), where he has figured the various types of lagging bivalents and stages in their separation. From this it would appear that the condition most conducive to normal disjunction of the components of a bivalent is the complete terminalization of chiasmata prior to metaphase I.

Secondary pairing. Secondary pairing may be described as pairing at metaphase resulting from a generalized attraction between bivalents related phylogenetically, though distantly. When the two chromosomes of a bivalent are held together at diakinesis and metaphase I by chiasmata, and each chromatid is condensed into a spiral coil, their particulate attractions which led to their pairing at zygotene are released. They may then be exercised on any similar particles within their spheres of influence. But the attachment constrictions appear to be the seat of localized forces leading to their mutual repulsion. The attractive forces appear to diminish more rapidly with increasing distance from a particular chromosome than does the repulsive force. Hence, the sphere of attraction appears to be relatively limited, and it can only be effective when the bivalents are more or less closely approximated. This condition is fulfilled at prometaphase, when the bivalents are massed together into a close group. Subsequently the unpaired bivalents and groups of secondarily paired bivalents repel one another mutually, so that they become more or less isolated on the metaphase plate, the secondarily paired bivalents remaining in close association. The actual response of attraction between related chromosomes must also depend upon their size, smaller bivalents being moved more easily than larger ones. It is because of this mechanical difficulty that secondary pairing is not found in polyploids with large chromosomes. Catcheside (1937) has shown that secondary pairing observed at metaphase between various bivalents disappears by mid-anaphase owing to the setting in of repulsion.

Since a sufficient number of polar view first metaphase plates was not available, the observations on secondary pairing had to be restricted to metaphase II, where it was equally well marked. There are several cases known in plants where secondary association of chromosomes was more marked at metaphase II than at the first (Müntzing, 1933, in *Solanum*; Nandi, 1936, in rice). Müntzing, attributes this to the cause

that at metaphase II the chromosomes are relieved of all the tension existing at the first division.

The extent of secondary pairing as seen in polar view of metaphase II is summarized in Table VIII, and the various types of association are shown in Table IX and Figs. 106-115. From Table IX it is clear that the

TABLE VIII

Polar view division II metaphase plate possessing the following number of secondary associations

	1	2	3	4	5	6	7	Total
Frequency	4	14	26	21	12	2	1	80

TABLE IX

Showing the types of secondary associations at metaphase II in Brassica monensis

No. of secondary associations	No. of chromosomes in association				Frequency	Totals
	1	2	3	4		
1	10	1	—	—	4	4
2	8	2	—	—	11	14
	9	—	1	—	3	
3	6	3	—	—	18	26
	7	1	1	—	8	
4	4	4	—	—	12	21
	5	2	1	—	8	
	6	—	2	—	1	
5	2	5	—	—	4	12
	3	3	1	—	7	
	4	2	—	1	1	
6	3	2	2	—	1	2
	2	3	—	1	1	
7	1	2	1	1	1	1
					Total 80	

maximum number of secondary associations is seven, which has been observed only once in eighty cells. At this apparent maximum the total number of bodies in the plate is five, which is therefore the basic number. The maximum type of secondary association observed in this species, viz. 1 (4)+1 (3)+2 (2)+1 (1) (Fig. 115), suggests that it is once octosomic, once hexasomic, and doubly tetrasomic. Hence the haploid chromosome set of the species can be represented as follows:

A A A A
B B B
C C
D D
E

(ii) *Brassica Wrightii* n. comb. ($2n=24$).

This is a wild species, most probably endemic to Lundy Island, from where it was first collected by Dr Ellison Wright. Prof. Schulz (1936) examined the dry material of this species and placed it in his genus (*Brassicella*) on the basis of the higher number of nerves on the pod, under the name *Brassicella Wrightii*. Cytologically it has the same chromosome number as *Brassica monensis* Huds. ($2n=24$) and has also been found to cross freely with *B. cheiranthus* Vill. ($2n=48$). Cytological observations, therefore, well justify retaining this species in the genus *Brassica*. The name, therefore, becomes *B. Wrightii* comb.nov. The latter name has been adhered to in the present description. The formation of twelve bivalents at diakinesis (Fig. 116) in this species confirms the somatic chromosome number, twenty-four, originally found by Gates.

Twelve bivalents at this stage is, however, a rare condition, and instead various multivalent associations ranging from a quadrivalent to a decavalent were observed in different nuclei. Fig. 117 shows two quadrivalents and eight bivalents, Fig. 118 one sexivalent, one chain of four and seven bivalents, Fig. 119 one octovalent and eight bivalents, Fig. 120 one decavalent and seven bivalents. The various shapes assumed by these multivalent configurations have been illustrated separately in Fig. 112 A-L. From these illustrations it is obvious that sometimes more than one chromosome is attached to one end of another chromosome. The probable explanation for this condition is that the chromosome threads must have been split prior to synizesis, that is, they consisted at that early stage of two chromatids each (Meurman, 1929). Only by the assumption that the four ends of one chromosome have each been attached to a different homologous chromosome, can such a pairing be explained.

Table X gives the frequency of different multivalent associations in thirty well-fixed nuclei analysed at late diakinesis. Besides the multivalent configurations mentioned in the table, in one nucleus all the chromosomes excepting two bivalents were seen to be linked together to produce a k-shaped figure (Fig. 121).

Presuming five as the correct basic number for *Brassica*, the constitution of this species may be represented by the formula $4b+4$. On the basis of this constitution, therefore, formation of multivalents up to an octovalent is quite possible in this species, provided regular pairing and chiasma formation occurs between the eight similar chromosomes in the complement. But as we know chiasma formation between homologous chromosomes is fortuitous, multivalent associations of lower order

than octovalent should be more frequent. Table XI reveals this actually to be the case as 40% of the nuclei showed only a quadrivalent. The formation of associations higher than octovalent is probably to be explained on the assumption that reciprocal translocations between non-homologous chromosomes have also occurred. This assumption is justified by the fact that occasionally chromosomes of different sizes are associated, as is clear from Figs. 120 and 122. Several other plants are known in which high multivalent formation has been recorded. Meurman (1929) in *Prunus Laurocerasus* observed multivalent associations ranging from trivalents to septivalents, and concludes that these are the result of real synaptic pairing of chromosomes at prophase. In the quinquevalent, sexivalent and septivalent groups, he observed that often three and sometimes four chromosomes were attached to one chromosome—a

TABLE X

No. of chromosomal bodies in the nucleus	Constitution of the various configurations	Frequency
7	$1_V + 1_{IV} + 5_{II}$	1
8	$1_V + 7_{II}$	2
9	$1_{VI} + 1_{IV} + 7_{II}$	1
	$1_{VIII} + 8_{II}$	3
10	$2_{IV} + 8_{II}$	2
	$1_{VI} + 9_{II}$	4
11	$1_{IV} + 10_{II}$	12
12	12_{II}	5

situation similar to that described in the present species. Lawrence (1929, 1931a) in *Dahlia variabilis* ($2n=64$)—an octoploid species—observed marked multiple associations at both first and second divisions, the degree of association varying from quadrivalents to octovalents. Darlington (1930) has figured multivalent associations up to sexivalents in some supposedly diploid forms of *Prunus*. In the pentaploid *Tulipa clusiana* (Newton & Darlington, 1929) all valencies up to quinquevalents have been observed. Srinath (1939) observed a frequent association of chromosomes, ranging from trivalents to a group of eight, in *Calceolaria mexicana*, an aneuploid species with $n=30$ chromosomes.

As the plant from which the material for the present study was collected produced only a few buds, unfortunately no pollen mother cells showing metaphases were obtained. Hence observations on the behaviour of multivalent bodies at this stage could not be made. However, judging from the later meiotic stages, which were quite regular, it can be safely inferred that the orientation of the multivalent configurations on the equator of the spindle must have been regular. Lawrence (1929) in

Dahlia variabilis and Darlington (1930) in some diploid forms of *Prunus*, observed a similar regular meiotic process in spite of the fact that high multivalent associations of chromosomes were very frequent in those plants.

Another interesting feature in this species is the presence of relatively inverted segments in a single pair of chromosomes, which leads to the formation of a chromatin bridge at anaphase I (Fig. 123). In the figure the bridge is seen to be joining the two poles and is of unequal thickness. The thick and thin portions of the bridge indicate that it is under great tension, probably due to axial stretching of the spindle, which has been regarded by Belar (1929*a*), Darlington (1937) and Alam (1936) as an important factor in causing the anaphasic separation of the chromosomes. Owing to this tension the bridge may break at any point. No fragment was detectable in this cell.

The presence of relatively inverted segments of chromosomes has been cytologically demonstrated in a number of plants and animals by the study of various meiotic stages. In the prophase stages (diplotene and pachytene) the chromosomes having such inverted segments have been seen to pair by the formation of a characteristic loop (McClintock, 1931, in *Zea*; Darlington, 1930, in *Chorthippus* and *Stauroderus*; Upcott, 1937, in *Tulipa*; Koller, 1936, in salivary chromosomes of race hybrids of *Drosophila pseudo-obscura*; Parthasarathy, 1939, in *Phalaris brachystachys*). At metaphase, however, the presence of inverted segments is difficult to demonstrate, unless the individual chromatids of the bivalent concerned remain separate (Dark, 1936, in *Paeonia*). At anaphase the inverted segments are shown more conclusively by the formation of a chromatin bridge owing to one or more cross-overs having occurred in the inverted region. The significance of the occurrence of the bridge and fragment at anaphase was first inferred by McClintock (1933) in irradiated *Zea mays* as cytological evidence for crossing-over in the inverted segment. Richardson (1936) has given a full analysis of the various types of cross-overs that can occur in the inverted region as well as proximal to it, in relation to the different kinds of bridges formed. Only those relationships which are relevant to the present study will be mentioned here.

A first division bridge (with two centromeres) and a fragment (with no centromere) can arise under any of the following three circumstances: (i) a single chiasma in the inversion, (ii) two chiasmata in the inverted region, one chromatid being involved in both the cross-overs, (iii) one cross-over in the inversion and one proximal to the inversion, the

chromatid relationship in this case being (a) the same two chromatids are involved in both the cross-overs, (b) the chromatids involved are different in the two cross-overs.

A second division bridge arises from two cross-overs, one in the inversion and the other in the region proximal to it, in which only one chromatid is involved in both the cross-overs. With chiasmata of the above relationship a monocentric loop and a fragment would be formed at anaphase I. The loop chromatid forms a bridge at anaphase II when the centromere divides (Smith, 1935). The fragment may or may not survive the first division.

The size of the fragment is considered by Darlington (1937) to be a fair measure for judging the size of the inversion. If the inversion is very near the centromere, the fragment is larger in size. Since the size of the fragment is in inverse proportion to the bridge, the position of the inversion can be inferred from the size of the fragment. Sax (1937), however, considers that fragments of different sizes may result from the same inversion by the occurrence of inverted cross-overs in non-homologous straight association of inverted parts, or by crossing-over in loop-pairing, which may shift throughout the length of the chromosome arm, extending to the non-homologous regions as well. In the present species, as no fragments were observed, this sort of analysis is impossible. However, the rare occurrence of the bridge indicates that the length of the inverted segment is very small, so that frequent formation of chiasmata in that region is not possible. Under such circumstances the inverted segment will pair straight with normal segment (non-homologous association) by torsion, as shown conclusively by McClintock (1933) in *Zea*. The genetical results of this suppression of crossing-over will be dealt with later.

(iii) *Brassica sinapistrum* Boiss. ($2n=18$).

This is a wild species native probably of southern Europe, but at present it is one of the most abundant weeds of cultivation throughout Europe and Asia, being too common all over Britain (Bentham & Hooker's *Flora*). The somatic chromosome number is found to be eighteen (Fig. 124). As the root-tips were fixed only in Navashin's fluid the number of satellitic chromosomes in the complement could not be ascertained.

At diakinesis and metaphase I nine bivalents are formed in the large majority of nuclei (Figs. 125, 127). Occasionally a ring of four chromosomes was observed at diakinesis (Fig. 126). The ring of four being very rare, its formation due to segmental interchange between non-homologous

chromosomes is a very remote possibility. Most probably the ring results from occasional pairing and chiasma formation between the four duplicated chromosomes present in the complement, which have presumably become structurally dissimilar and are homologous only over short lengths. Occasionally two univalents were also observed at metaphase (Fig. 128).

At anaphase I the disjunction of the bivalents is mostly normal, but in one pollen mother cell a distribution of ten and eight chromosomes was observed (Fig. 129). This irregular distribution was most probably caused by the non-disjunctional arrangement of the ring of four chromosomes on the spindle, which leads to its division into three and one instead of two and two. From such gametes could arise new individuals with $2n+1$ or $2n-1$ chromosomes. Since gametes and xygotes with extra chromosomes are viable more often than those lacking certain chromosomes, hyperploid types are likely to arise more frequently than hypoploid ones. The occurrence of non-disjunction in a pure species like this becomes more significant when we consider that the whole genus *Brassica* is characterized by heteroploid numbers of chromosomes.

Non-disjunction was first recorded by Gates (1908) in pollen mother cells of *Oenothera rubrinervis*. Later, Davis (1910, 1911) observed it in *Oe. biennis* and *Oe. Lamarchiana*. The significance of non-disjunction in relation to the origin of trisomic mutations was soon recognized (Gates, 1923). Trisomic mutations have since been recorded in a large number of plants and animals, e.g. *Drosophila* (Bridges, 1921), *Datura* (Blakeslee, 1922, 1928, 1931) and *Crepis* (Navashin, 1926, 1930; Hollingshead, 1930). The later meiotic process in this species is quite normal, no irregularities have been observed.

The secondary pairing of chromosomes was studied at metaphase II and the results obtained have been summarized in Tables XI and XII. The various types of secondary association are illustrated in Figs. 130-136. From Table XI it will be noticed that four is the maximum

TABLE XI
*Showing the extent of secondary association of univalents in
polar view metaphase II plates*

	No. of secondary associations					Total
	0	1	2	3	4	
Frequency	8	10	19	6	2	45

number of secondary associations, although the mode occurs at two. The types of secondary associations at the apparent maximum point is a

little variable, namely, four groups of two bivalents (Fig. 136), one group of three with two groups of two bivalents (Fig. 135). The formation of a group of three is probably to be explained on the basis that two of the chromosomes are structurally changed, resulting in different distribution of homology. These structurally changed chromosomes would have a weak affinity for each other. Now if one of these had already paired with its mate to give a group of two, and the other one happened to be away from the chromosome for which it has greater affinity, but within the range of attraction of its weakly homologous chromosome, it might pair with the latter to give a group of three.

TABLE XII
*Showing the types of secondary association at metaphase II
in Brassica sinapistrum*

No. of secondary associations	No. of chromosomes in associations			No. of cases	Totals
	1	2	3		
0	9	—	—	8	8
1	7	1	—	10	10
2	5	2	—	16	19
	6	—	1	3	
3	3	3	—	3	6
	4	1	1	3	
4	2	2	1	1	2
	1	4	—	1	

Total 45

If the secondary pairs of univalents are each considered as one body, then the number of bodies in a plate has never been found to be less than five, indicating that five is the basic number. On the basis of maximum association observed, viz. 4 (2) + 1 (1), the haploid chromosome set of *Brassica sinapistrum* (assuming that structural changes have occurred) may be represented as follows:

A A
B B
C C
D D
E

(iv) *Brassica nigra* Koch ($2n = 16$).

This is a widespread species, occurring in central and southern Europe and temperate Asia. In India and Tibet it is cultivated for its seeds. It is also found scattered over England and is apparently wild on some points of the south coast. Bentham and Hooker think that it has been introduced into Britain.

At diakinesis eight bivalents are observed* in most of the nuclei (Fig. 137). The bivalents are well spread out in the nucleus, which indicates repulsion between them. Two bivalents are found attached to the nucleolus at this stage (Fig. 139). These would correspond to the four SAT-chromosomes observed in the somatic tissue. The forms of bivalents are various, and regular size differences similar to those existing in somatic chromosomes may be seen. Although the chromosomes at the earlier stages of prophase are too small for critical study, yet judging from their later history it is evident that chiasmata are established interstitially and undergo terminal movement. Opening out of the loop containing the attachment constriction is usually carried to its extreme limit, with the result that the two chromosomes of a bivalent are held together by terminal chiasmata, either one or two in number. Hence both ring and rod bivalents result. But all the chiasmata do not terminalize fully, and they are, therefore, more or less interstitial at diakinesis and persist as such till metaphase I (Fig. 141).

Although the formation of eight bivalents at diakinesis is a general feature of this species, formation of a quadrivalent besides six bivalents is not uncommon. The quadrivalents observed were either rings of four chromosomes (Fig. 138) or consisted of two bivalents linked end to end by a very fine thread (Fig. 139). The formation of quadrivalents in this species is quite consistent with its allotetraploid nature, which has already been inferred from its somatic chromosomes.

In a few extreme cases linkage of more than two bivalents was also observed. In Fig. 140 four bivalents are seen associated by slender threads, which are very much drawn out owing to repulsion between the bivalents that are characteristic of diakinesis. Another noteworthy feature in this connexion is the linkage of separate bivalents to the same end of the third bivalent. The probable explanation for such a condition has already been advanced in case of *B. Wrightii*, in which a similar phenomenon was observed. Haga (1938), in a study of meiosis of this species, did not observe any multivalent configurations at diakinesis or later stages. Perhaps he did not observe a sufficient number of pollen mother cells, as the frequency of quadrivalent formation is very low.

Considering that *B. nigra* has the lowest chromosome number known in the genus it cannot be regarded as a high polyploid, and therefore the formation of an octovalent, described above, will have to be explained on the hypothesis of segmental interchange. By reciprocal translocations non-homologous chromosomes can come to possess homologous segments at their ends, chiasma formation between which would result in multi-

valent configurations. That segmental interchange has occurred in this species is further supported by the fact that in metaphase I we frequently see high secondary association of chromosomes (Figs. 148, 149). The only explanation for end-to-end linkage of whole bivalents rather than single chromosomes, seems to be that the length of the middle homologous portions of the components of a bivalent is much more than that of the interchanged end portion. This allows the formation of more than one chiasma in the former region, while only a single chiasma is formed in the latter. Thus the frequency of multivalents would depend upon the frequency of chiasma formation in the interchanged region. If the end homologous portions are too small to allow frequent chiasma formation, only bivalents would result. Perhaps the very low frequency of multivalents in this species is due to this cause.

The association of chromosomes in groups is characteristic largely of autopolyploids, but it can also occur in allotetraploids derived by doubling the chromosome complement in a structural heterozygote, provided the chromosomes of such a heterozygote have undergone segmental interchange, which allows pairing of homologous parts between structurally dissimilar chromosomes. Such allopolyploids, as is the case with autopolyploids, have no differentiation between the corresponding chromosomes that would seriously interfere with their pairing at meiosis, thus making high associations possible.

With the disappearance of the nuclear membrane the chromosomes, which have already become smaller in size owing to further contraction, aggregate in the centre of the nucleus (pro-metaphase). They are, however, soon spread out again owing to body repulsion of chromosomes, and come to lie on the equator. Both in polar and side views of metaphase all the chromosomes, whatever their physical nature may be, were observed to form a regular plate, excepting a few rare cases where one or two bivalents did not congress on the plate (Fig. 152). The causes of non-congression and non-orientation will be discussed later.

In the side view of metaphase the multivalent configuration observed at diakinesis could not be made out. This is largely due to the fact that the connecting threads, which are very slender and of tenuous nature, lie obliquely to the plane of the slide, thus making the discernment of the physical continuity among the individual components of multivalent associations impossible. The situation is further aggravated by the overlapping of the chromosomes on the metaphase plate. In polar view also, although the connexions between the paired chromosomes lie for the most part axially in the spindle and are, therefore, invisible, the con-

nexions can be inferred from the juxtaposition of the chromosomes. Fig. 142 shows one quadrivalent and six bivalents, while in Fig. 143 one sexivalent and five bivalents have been illustrated. Both these kinds of multivalents were seen only once in about fifty cells examined at metaphase I.

Secondary association between bivalents was quite marked in polar views of metaphase I. Table XIII shows the types of association observed in forty-one well-differentiated plates. The various types of secondary association have been illustrated in Figs. 144-149.

TABLE XIII

Showing the types of secondary associations at metaphase I in Brassica nigra

No. of secondary associations	No. of bivalents in association							Frequency	Total
	1	2	3	4	5	6	7		
1	6	1	—	—	—	—	—	2	2
2	4	2	—	—	—	—	—	16	20
	5	—	1	—	—	—	—	4	
3	2	3	—	—	—	—	—	8	14
	3	1	1	—	—	—	—	6	
4	1	2	1	—	—	—	—	1	1
5	1	1	—	—	1	—	—	2	2
6	1	—	—	—	—	—	1	2	2
Total 41									

The observed kinds of secondary pairing in this species fall into six classes. An inspection of the frequencies of the various classes suggests that the first three are normal and characteristic, and the remainder aberrant. The aberrant classes appear to be due to real structural complexity of the species, which is also evident from the high multivalent associations observed at diakinesis and metaphase I. Haga (1938) observed similar aberrant classes in his material, which he explains as due to superimposed structural changes of certain chromosomes. The characteristic maximum also shows two types of pairing, viz. 3 (2) + 2 (1) (Figs. 145, 146) and 1 (3) + 1 (2) + 3 (1) (Fig. 147). The reasons for the formation of the group of three bivalents appear to be the same as already advanced in case of *B. sinapistrum*. On the whole, the data agree well with the hypothesis that the basic chromosome number is five; on the basis of the secondary pairing of the type 3 (2) + 2 (1), which appears to be the normal kind, the haploid chromosome set of this species may be represented as follows:

A A
B B
C C
D
E

Cytomixis. Another interesting feature noticed in this species was the partial transference of chromatin from the nucleus of certain pollen mother cells to the cytoplasm of the contiguous cells through gaps in the cell walls. This peculiar extrusion of chromatin was seen both at metaphase I (Fig. 150) and interkinesis (Fig. 151). Whether these extrusions occurred actually at these stages or in early thread stages is difficult to decide, although the latter seems more probable. Kattermann (1933) thinks that the extrusion of chromatin at stages later than prophase is not possible, because the cytoplasm of the pollen mother cell changes from a sol at prophase to a gel at later stages. Church (1929) has, however, described the various types of grasses displaying different kinds of cytomixis. The first type are those where the phenomenon is seen only in the "spireme" stage, but with the resultant extrusion persisting in later stages. The second type exhibits such chromatin loss to the extent that the process is still manifested at diakinesis. The third type is that where it is observed in abundance at diakinesis.

Gates (1911) was the first to observe the transference of chromatin from one pollen mother cell to another at prophase in *Oenothera gigas*, and suggested the term "cytomixis" for this phenomenon. Later, Gates & Latter (1927) observed cytomixis in *Lathraea* during interkinesis. Katterman (1933) shows various stages of cytomixis in pollen mother cells of *Triticum* \times *Secale* hybrids and gives references to all the earlier work on the subject. Kihara & Lilienfeld (1934) described it in *Triticum* \times *Aegilops* hybrids, where the phenomenon was so marked as to give rise to binucleate pollen mother cells by the complete transference of the chromatin of one nucleus to the adjoining cell. Nandi (1936) observed a similar condition in rice. Cytomixis has also been described by Bhatia (1938) in *Triticum* and Iyengar (1939) in *Cicer*. As far as the author is aware, cytomixis in *Brassica* has not been previously reported.

Non-orientation and non-congression of bivalents. As mentioned earlier, one or two bivalents were seen occasionally to lie off the metaphase plate in this species. But judging from the regular anaphase behaviour of chromosomes and the absence of more or less than eight univalents in metaphase II plates, it is evident that these bivalents orientate themselves on the equator later and divide regularly. Darlington (1937) attributes the non-congression of the bivalents to body repulsion of other chromosomes on a crowded plate, and the delay in orientation of bivalents to increased distance between the centromeres, with consequent decrease in repulsion. He further thinks that repulsion is the effective agent in orientation. On his hypothesis, therefore, rod bivalents should be more

subject to non-orientation than ring bivalents. But this is not always the case. In the present species both types of bivalents seemed equally liable to late orientation. Parthasarathy (1939) observed a similar phenomenon in certain species of *Phalaris*, and thinks that the delay in orientation is caused by the disposition of the axis of the centromeres in the bivalents during the course of disappearance of nuclear membrane and the determination of the poles. Those bivalents whose axes are in the direction of the poles move quickly to the equator, while those whose axes are perpendicular are delayed. In the present species Parthasarathy's hypothesis seems quite applicable, as is evident from Fig. 152, where one bivalent is seen lying crosswise in the spindle. The other bivalent in this figure seems to have rotated later so as to align the axis of its centromeres in the direction of the poles.

5. DISCUSSION

SPECIES FORMATION IN *BRASSICA*

In the genus *Brassica* six species (*B. campestris*, *B. rapa*, *B. chinensis*, *B. japonica*, *B. pekinensis* and *B. trilocularis*) are known, having the same chromosome number ($2n=20$) but exhibiting wide variations in the morphological characters of the plant. Most of these species cross with each other freely, producing fertile hybrids (Morinaga, 1928; Richharia, 1937b; and one hybrid described in this paper). Some of these hybrids have been studied genetically and have been found to obey Mendelian laws of segregation (Ali Mohamad & Sikka, 1938). The differences between these species, like those of *Antirrhinum* (Baur, 1932), can, therefore, be regarded as of the nature of simple gene mutations.

In conjunction with such genic or genotypic changes, chromosomes are subject to another kind of change described as structural or numerical according as it affects the structure of the chromosomes or their number. From the cytological standpoint, the changes in number include (a) polyploidy, (b) polysomy. Structural changes include (i) segmental interchange, (ii) duplication, (iii) inversion, (iv) deletion of a portion of a chromosome (cf. Gates, 1938). Some of these changes involve no immediate phenotypic change in the organism, but they serve as a basis on which future differentiation of types can take place.

Of these changes, numerical variation seems to be very much favoured in nature, as is shown by the fact that at least half the species and varieties of flowering plants belong to a polyploid series. The nature of the duplicated sets determines the kind of polyploidy. The genus *Brassica*, consisting of species with ($2n$) chromosome numbers 16, 18, 20, 22, 24,

36, 38 and 48, belongs to the allopolyploid class. Cytology has provided most reliable clues to the different methods which are instrumental in the production of polyploid species, but our knowledge of the origin of species with aneuploid numbers has been meagre up to the present time. There being no direct source of evidence, attempts have been made to solve the phylogenetic relationships of such species by indirect means. The best source of evidence in these cases has been the secondary association of chromosomes at meiosis, which manifests itself as a generalized attraction between chromosomes phylogenetically, though distantly, related. It appears to be due to a residual attraction between chromosomes in which ancestral homology is still discernable, and is, therefore, an indication of polyploidy. Lawrence (1931b), who has given a general review of the problem, considers that secondary association is a phenomenon intimately connected with allopolyploidy, and that the degree of association can be used as a measure of the phylogenetic age of the form under examination. This type of study has already proved fruitful in many plant genera in providing reliable clues with regard to phylogeny. As examples may be quoted *Oryza* (Sakai, 1935; Nandi, 1936; Ramanujam, 1938), *Pomoideae* (Darlington & Moffett, 1930), *Dahlia* (Lawrence, 1931), *Gossypium* (Davie, 1933; Skovsted, 1937), *Cicer* (Iyengar, 1939) and *Calceolaria* (Srinath, 1939).

From the study of secondary association of chromosomes in three species of *Brassica* reported in this paper, the conclusion seems justified that the basic number for the genus is five. The correctness of this basic number may perhaps be shown by comparing the secondary chromosome numbers of *Brassica* with the numbers found in related genera of Cruciferae. Manton (1932) has recorded the chromosome numbers of three species of *Erucastrum*—a genus belonging to the same subtribe as *Brassica*—as thirty and of two other species as thirty-two. Manton thinks that species with thirty chromosomes have been derived from those with thirty-two by loss of two chromosomes. To me it appears that these species are hexaploid on the original basic number five, while the species with thirty-two chromosomes may have arisen through reduplication of two chromosomes. A still more remarkable example is afforded by the genus *Crambe* of the subtribe Raphaninae. The chromosome numbers in this genus form a regular polyploid series, viz. 30, 60, 90, 120, which are all multiples of thirty, itself a multiple of five. It therefore seems reasonable to believe that while some allied genera of Cruciferae retained the number five as a basis for polyploidy and others later used thirty as a basis for still higher multiples, the Brassiceae

developed a wide range of secondarily balanced numbers by intercrossing. An analysis of the chromosome numbers in *Brassica* itself is also indicative. Of the nineteen species, of which chromosome numbers are known, seven show twenty as the diploid number, three have $2n=18$, two $2n=24$, only one has $2n=16$ and the remaining six species with higher chromosome numbers have been shown to be amphidiploid derivatives of these. It is, therefore, evident that a large majority (nearly 50%) of the species in the genus have retained the tetraploid number (4b) on the basis of five. The other few with a slightly higher or lower number must have resulted by reduplication or loss of certain chromosomes from the 4b number. Thus, while some genera of Cruciferae related to *Brassica* developed hexaploidy, this genus has developed tetraploidy.

Catcheside (1934), from the study of secondary association of chromosomes in *B. napus*, concludes that six is the primary basic number for the genus *Brassica*. In support of this he adduces evidence that the primitive haploid number in Cruciferae is seven, from which six—the alleged basic number for *Brassica*—could be derived by fusion of two chromosomes. Manton (1932), however, thinks that the data for assuming seven as the haploid number of Cruciferae are quite inconclusive. Moreover, as the chromosome number of *B. napus*, as originally determined by Catcheside, has been proved to be erroneous by Howard (1938), this species requires re-examination before coming to any final conclusion. In *B. oleracea* also Catcheside (1937) concludes that the primary basic number is six, although he observed several cases of four groups of two and one single, i.e. five groups in all, both at metaphase I and metaphase II. He thinks that this type of pairing is due to some superimposed structural changes.

Alam (1936), from a study of three species of *Brassica*, arrives at the same basic number as Catcheside. The disparity between Alam's observations and those recorded in the present paper may possibly be explained by the fact that the forms examined by him may be of very remote origin, so that the structural differentiation of some homologous chromosomes has occurred to such an extent that their affinity is no longer enough to cause attraction. Under these circumstances, maximum secondary pairing of chromosomes equal to the true basic number would be only a very remote possibility. In any case, a re-examination of these forms also seems necessary to clarify the position further.

Besides the cytological basis which has shown secondarily balanced polyploidy to be present in *Brassica*, genetic findings of several authors have shown the presence of duplicate and triplicate factors as well as

linkage groups each containing two or more duplicate factors in certain species. These genetical data are, therefore, an extra proof of the polyploid nature of these forms. Moreover, the incompatibility interrelationships in the cabbage, determined by Detjen (1927) and Kakizaki (1930), are also such as is characteristic of polyploids. This question has been discussed at length by Lawrence (1930).

Still further evidence for the polyploid nature of *Brassica* species may be obtained from the number of satellitic chromosomes and nucleoli in the somatic complement. *B. nigra*, which has the lowest chromosome number in the genus, has been shown to possess four satellites and four nucleoli. This condition, therefore, strongly suggests the tetraploid nature of the species. On this basis other forms of *Brassica* having higher chromosome numbers, viz. $2n=18, 20, 24$, must also be regarded as tetraploids, although these forms have shown so far only one pair each of satellites and nucleoli. Their condition evidently suggests the subsequent loss of the extra pair of satellites through mutation, as has been inferred in other cases. Navashin (1926, 1934) found in *Crepis* crosses that a satellite normally present in one of the contributing complements did not appear in the hybrid. This phenomenon, termed amphiplasty by him, may be interpreted as due either to the inability of the nucleolar body to function in a new environment or to competition in the nucleolar organization by the nucleolar chromosomes, resulting in the apparent inability of one of them to function. Such a phenomenon has a direct bearing on allopolyploidy. If the hybridity has an amphiplastic effect in the suppression of a pair of satellites, the organization of the nucleolus is also suppressed and two nucleoli instead of four may result. In rice, Ramanujam (1937 a) has inferred another type of change in the evolution of varieties with two from varieties with four nucleoli. A mutation leading to the inactivation of the nucleolar organizer or a loss of satellites in varieties with four will give in subsequent generations varieties with four and two nucleoli, respectively. In a study of the chromosomes of *Crepidinae*, Babcock *et al.* (1937) figure the idiograms of many species of *Lactuca*, four of which show four SAT-chromosomes each, while one has only two. Evidently the last species must have lost one pair of satellites in the course of evolution. In *Brassica*, further studies on other 18-, 20-, 24-chromosomal species may perhaps bring to light a condition similar to that in *Lactuca*. It is quite probable that some of these species may still have retained the duplicated complement of satellites.

Based on the study of interspecific hybrids of *Brassica*, various workers have shown the existence of three genomes termed *A*, *B* and *C*,

consisting of $n=10$, 8 and 9 chromosomes, respectively. That these various genomes have arisen from a common origin may further be proved by the fact that they exhibit a weak affinity towards each other, as is clear from the following instances:

(i) When *B. campestris* ($2n=20$, *AA*) is crossed with *B. oleracea* ($2n=18$, *CC*) the F_1 hybrid ($2n=19$, *AC*) shows $(0-8)_{II} + (19-3)_I$ at metaphase I (U, 1935), which shows a definite affinity between genomes *A* and *C*. Similarly, in the cross *B. napus* ($2n=38$, *AACC*) \times *B. oleracea* (*CC*) the F_1 ($2n=29$, *ACC*) shows $(0-5)_{III} + (9-4)_{II} + (10-5)_I$. Evidently the trivalent formation is due to conjugation of certain chromosomes of *C* genome with those of *A*, which must then be homologous.

(ii) When *B. carinata* ($2n=34$, *BBCC*) is crossed with *B. oleracea* (*CC*) the F_1 ($2n=26$, *BCC*) shows $(9-5)_{III} + (0-4)_{II} + (8-12)_I$ (U, 1935). These results indicate a definite affinity between *B* and *C* genomes.

(iii) When *B. juncea* ($2n=36$, *AABB*) is crossed with *B. campestris* ($2n=20$, *AA*), as described elsewhere in this paper, the F_1 ($2n=28$, *AAB*) shows occasionally a quadrivalent at metaphase I, due evidently to pairing between homologous chromosomes of the genomes *A* and *B*.

(iv) The haploid of *B. napella* ($2n=38$, *AACC*) shows 0-6 and sometimes seven bivalents at meiosis (Morinaga & Fukushima, 1933).

Several instances are known in the plant kingdom, where secondarily balanced polyploids have been experimentally produced by the addition of certain chromosomes of one species to those of another. Clausen (1926) obtained a new "species" *Viola hyperchromatica* from the cross *V. arvensis* ($2n=34$) \times *V. tricolor* ($2n=26$), having approximately 42-45 chromosomes. Collins *et al.* (1929) obtained *Crepis artificialis* from the cross *C. biennis* ($n=40$) \times *C. setosa* ($n=4$). The new form was fairly constant, containing ten pairs of *biennis* and two pairs of *setosa* chromosomes. Lammerts (1932) obtained a new fertile type with sixty chromosomes from the cross *Nicotiana rustica* ($n=24$) \times *N. paniculata* ($n=12$). This type bred true through eight generations of selfing.

Another line of evolution obvious in the genus *Brassica* is the establishment of amphidiploid forms with higher chromosome numbers through further hybridization of the various 16-, 18-, 20- and 24-chromosomal species. This fact has been proved beyond any doubt by genome analysis of five such species, the results of which are summarized in Table XIV. The most remarkable case is that of *Brassica napus* ($2n=38$), which has been synthesized by U (1935) from a cross between *B. campestris* ($2n=20$) and *B. oleracea* ($2n=18$). This experimentally produced amphidiploid had all the characters of the naturally occurring *B. napus*. On the basis

of this, therefore, it seems logical to believe that other supposedly amphidiploid species of *Brassica* must also have originated in nature in the same way. The highest chromosome number known in the genus is that of *B. cheiranthus* ($2n=48$). This species invariably shows 24_{II} at the heterotypic metaphase (Fig. 153). On this basis it appears to be allotetraploid, derived by doubling of chromosomes of a hybrid between two species each with $n=12$ chromosomes, such as *B. monensis* and *B. Wrightii*. This view seems justified on the following grounds also, although the final decision of this question will have to be postponed till a study of hybrids between this species and its supposed parents has been under-

TABLE XIV

	Cross	Authority
(i) <i>B. carinata</i> ($n=17$) × <i>B. oleracea</i> ($n=19$)	$9_{II} + 8_I$	U (1935)
(ii) <i>B. carinata</i> ($n=17$) × <i>B. nigra</i> ($n=8$)	$8_{II} + 9_I$	Do.
(iii) <i>B. juncea</i> ($n=18$) × <i>B. campestris</i> ($n=10$)	mostly $10_{II} + 8_I$ rarely $1_{IV} + 9_{II} + 6_I$	Author
(iv) <i>B. juncea</i> × <i>B. nigra</i>	$8_{II} + 10_I$	Morinaga (1934)
(v) <i>B. napus</i> ($n=19$) × <i>B. campestris</i> ($n=10$)	$10_{II} + 9_I$	U (1935)
(vi) <i>B. napus</i> ($n=19$) × <i>B. oleracea</i> ($n=9$)	$(0-5)_{III} + (9-4)_{II} + (10-5)_I$	Do.
(vii) <i>B. napella</i> ($n=19$) × <i>B. campestris</i> ($n=10$)	$10_{II} + 9_I$	Morinaga (1929a)
<i>B. cernua</i> ($n=18$) × <i>B. campestris</i> ($n=10$)	$10_{II} + 8_I$	Morinaga (1929b)

taken. A cross of this species with *B. Wrightii* has already been obtained and is under study:

(i) *B. monensis*, *B. Wrightii* and *B. cheiranthus* overlap in geographical distribution.

(ii) The phenotypic characters of *B. cheiranthus* are midway between those of *B. monensis* and *B. Wrightii*.

(iii) *B. cheiranthus* is more vigorous and later in flowering than the other two species.

The process of evolution in the genus *Brassica* may be diagrammatically represented as in Diagram 3. Whether the known amphidiploids in *Brassica* have originated by the union of unreduced gametes or by somatic or gametic doubling is difficult to decide. In other plants all these methods have been found to be operative in the production of amphidiploids. *Primula kewensis* (Newton & Pellew, 1929), *Rosa Wilsoni* (Blackburn & Harrison, 1924), *Nicotiana glauca* (Clausen & Goodspeed, 1925), *Solanum nigrum-luteum* (Jørgensen, 1928), *Saxifraga Potternensis*

(Whyte, 1930), *Brassica napocampestris* (Frandsen & Winge, 1932) have originated by somatic doubling, whereas *Raphanobrassica* (Karpechenko, 1928, 1929), *Digitalis Mertonensis* (Buxton & Newton, 1928), *Nicotiana tabacum-sylvestris* (Rybin, 1929) are the result of fusion of unreduced

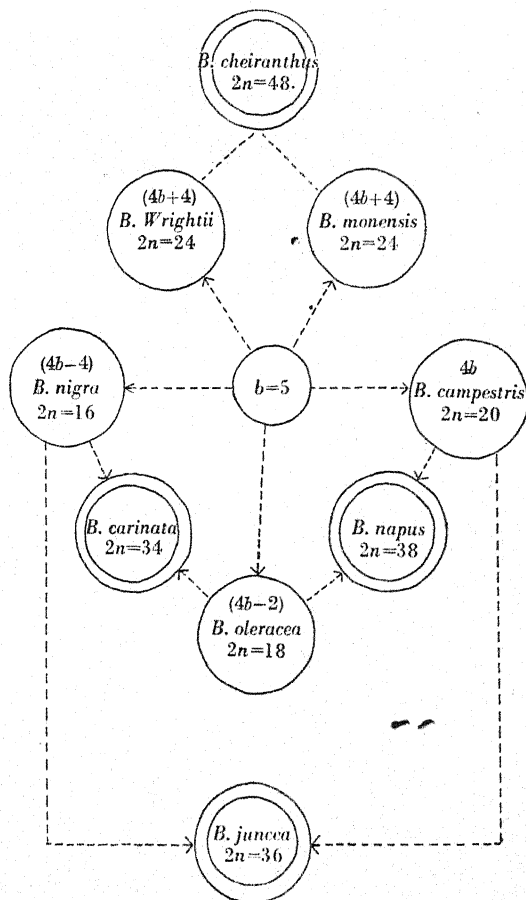


Diagram 3.

gametes. U (1935) thinks that artificially synthesized *Brassica napus* has arisen by somatic or gametic doubling and not by fusion of unreduced gametes.

Hybridization has, therefore, played the most important role in species formation in *Brassica*. Winge (1917) advanced the view that new species may arise through crossing of species, somatic doubling of the chromosomes entering into the production of the hybrid. His hypothesis

has since found ample support from a large number of plant genera. Allopolyploids seem more favoured in nature than autopolyploids. In an autopolyploid all the chromosomes of a genome being represented several times over, all the genes of the species are duplicated. The effect is collective intensification of characters. The spontaneous occurrence of structural changes being slow, the chances for new variations to arise in them are few compared with those in allopolyploids. Nevertheless the fertile autopolyploids with their new complements of chromosomes have greater potentialities for variation by gene mutations and structural changes than is possessed by the diploids. Allopolyploids have the added advantage of having genetically dissimilar chromosomes and the consequent capability of structural changes as a special mechanism of variation.

Of equally great significance are the structural changes of chromosomes as a mechanism of variation. As opposed to gene mutations their chief importance lies in effecting rapid evolutionary changes, provided the new types have a survival value. The ultimate effect of structural changes is that they inhibit pairing of the relatively changed chromosomes, which ultimately leads to intersterility. It was supposed until recently that interspecific sterility could only arise gradually over a long period and that it represented the final stage in the production of a true species. Recent experimental work has, however, proved the fallacy of this view by showing that intersterility is frequently the first stage rather than the last in the production of new species. A rearrangement of portions of the genome—an internal change which may have no phenotypic effect—has often occurred in one strain of a species, making it more or less infertile with the type. As a result of this intersterility, the new type as a fresh centre of breeding and mutations is placed on the road to becoming a new species (Gates, 1938).

Of all the structural changes to which chromosomes are liable, segmental interchange and inversions appear to be the most important. The universal importance of these two types of structural change can be realized from the fact that both ring-forming plants (e.g. *Rhoeo*, *Campanula*, *Humulus*, *Anthoxanthum*, etc.) and inversion heterozygotes occur in nature spontaneously. In *Drosophila*, inversions distinguish natural races (Sturtevant, 1926; Koller, 1935). The frequency of inversions is, therefore, a useful adjunct in the classification of races on a cytological basis. The direct factors concerned in effecting variations in the organism due to inversions are (1) the length of the reversed segment and (2) the frequency of crossing-over in it. Long inversions have a

deleterious effect on the organism as they allow frequent chiasma formation in the inverted segment, leading to bridge formation which results in an unequal chromatin distribution to the daughter cells and ultimate sterility. This accumulation of sterility will in the long run lead to complete suppression of sexual reproduction and the species has, therefore, to resort to vegetative reproduction for its existence. Short inversions, on the other hand, suppress crossing-over as they pair straight with corresponding uninverted segments by torsion (McClintock, 1933). Such a suppression of crossing-over in small inverted segments would result in the group of genes located in that region being inherited as a unit. Sturtevant & Dobzhansky (1936) have inferred the genetical suppression of crossing-over in certain regions of the *X*-chromosomes in *Drosophila* to be due to such inverted segments.

In *Brassica* both segmental interchange and inversions seem to have played an important role in species formation. The most remarkable example of the first type of change is afforded by *B. Tournefortii* and *B. trilocularis*. Although these species have the same chromosome number and individually form only bivalents at meiosis, the hybrid between them shows frequently a ring of four chromosomes. High multivalent formation in *B. nigra* and *B. Wrightii* is also partially due to reciprocal translocations. Inversions have been found in two hybrids (*B. juncea* \times *B. campestris* and *B. Tournefortii* \times *B. trilocularis*) as well as in a naturally occurring species (*B. Wrightii*). The fact that the last named species has a small inverted segment in one chromosome, while the allied species, *B. monensis*, with the same chromosome number ($2n=24$) does not show evidence of inversions is very significant.

6. SUMMARY

The cytological behaviour of three interspecific hybrids and twelve species of *Brassica* is described and the following are the chief results obtained:

1. The pairing of chromosomes in the F_1 cross *B. juncea* Coss. ($2n=36$) \times *B. campestris* L. var. *sarson* Prain ($2n=20$) takes place according to the Drosera scheme. With the exception of one cell, which showed $1_{IV}+9_{II}+6_I$, all the rest showed $10_{II}+8_I$. From this and other evidence the amphidiploid origin of *B. juncea* as a hybrid between *B. campestris* and *B. nigra* has been proved.

2. In the cross *B. Tournefortii* Gouan ($2n=20$) \times *B. trilocularis* H.f.T. ($2n=20$) the pairing of chromosomes is very variable. Nuclei with total lack of pairing to those showing a quadrivalent besides 1-3 bivalents

were observed. The whole meiotic process in the hybrid is very irregular, resulting in the formation of dyads, monads and tryads to the extent of 25, 8 and 5 %, respectively.

3. The conjugation of chromosomes in the hybrid *B. trilocularis* H.f.T. ($2n=20$) \times *B. rapa* L. ($2n=20$) is complete, 10_{II} being invariably formed at diakinesis and metaphase I. Non-disjunction of a bivalent was frequently observed at anaphase I. But for this irregularity the whole meiotic process is quite regular.

4. *B. sinapistrum* Boiss. has $2n=18$, *B. Tournefortii* Gouan $2n=20$, *B. monensis* Huds. $2n=24$ and *B. rugosa* $2n=38$ chromosomes.

5. Somatic chromosomes of nine species were examined, with special reference to the number of satellites and nucleoli. A complete correspondence between the two has been found.

6. Whereas *B. nigra* Koch, which has the lowest chromosome number in the genus ($2n=16$) shows four satellites and four nucleoli, *B. oleracea* L. ($2n=18$), *B. rapa* L. ($2n=20$), *B. campestris* L. ($2n=20$), *B. trilocularis* ($2n=20$) and *B. Tournefortii* ($2n=20$) show only one pair each. This suggests that the latter five species have lost the extra pair of satellites by mutation during the course of evolution. The presence of six satellites and six nucleoli in *B. juncea* and four nucleoli in *B. napus* and *B. rugosa* has been correlated with their amphidiploid nature, and is additional proof of this.

7. Other interesting features in somatic mitosis, such as somatic pairing, sporadic aberrations in chromosome numbers and formation of chromatin bridges, have been described.

8. From the secondary association of chromosomes in three species the primary basic number for the genus has been inferred to be five. This conclusion is supported by the chromosome numbers of some related genera in which the basic number five has been retained for polyploidy. In certain other genera the hexaploid number thirty is basic, and some species have become tetraploid on this basis. Some other evidence has also been adduced to show that all the monogenomic species of *Brassica* have a common origin.

9. While *B. campestris*, *B. trilocularis*, *B. Tournefortii* and *B. rapa* show exclusively bivalent formation at meiosis, *B. juncea*, *B. sinapistrum* and *B. monensis* show occasionally a quadrivalent. *B. nigra* and *B. Wrightii* show in addition higher multivalents. The probable reasons for the formation of multivalents in each of this species have been discussed.

10. Presence of relatively inverted segments of chromosomes has

been inferred in some species from the chromatin bridges formed at meiosis.

11. The role played by gene mutations, structural changes of chromosomes and hybridization in species formation in *Brassica* has been discussed. Of these hybridization has played the most important part, as amphidiploidy has frequently occurred in the genus.

7. ACKNOWLEDGEMENTS

This work was carried out under the direction of Prof. R. R. Gates, F.R.S., and I take this opportunity of recording my sincerest gratitude to him for his inestimable help, valuable criticism and unfailing kindness.

My grateful thanks are also due to H. R. Stewart, Esquire, Director of Agriculture, Punjab, Lahore, for relieving me of my official duties to undertake this study at the University of London.

REFERENCES

- AASE, H. C. (1930). "Cytology of *Triticum*, *Secale* and *Aegilops* hybrids with reference to phylogeny." *Res. Stud. State Coll. Wash.* **2**, 1-60.
- ALAM, Z. (1936). "Cytological studies of some Indian oleiferous Cruciferae. III." *Ann. Bot., Lond.*, **50**, 85-102.
- ANDERSON, E. G. (1935). "Chromosome interchanges in maize." *Genetics*, **20**, 70-83.
- BABCOCK, E. B., STEBBINS, G. L. & JENKINS, J. A. (1937). "Chromosomes and phylogeny in some genera of the Crepidinae." *Cytologia, Tokyo*, Fujii Jub. Vol. pp. 183-310.
- BARBER, H. N. (1938). "Delayed mitosis and chromatid fusion." *Nature, Lond.*, **141**, 80.
- BAUR, E. (1932). Cited from Gates (1938).
- BEADLE, G. W. (1930). "Genetical and cytological studies of Mendelian asynapsis in maize." *Mem. Cornell agric. Exp. Sta.* no. 129.
- (1931). "A gene in maize for supernumerary cell divisions following meiosis." *Mem. Cornell agric. Exp. Sta.* no. 135.
- BELAR, K. (1929a). "Untersuchungen an den Spermatocyten von *Chorthippus* (*Stenobothrus*) *lineatus* Panz." *Roux Arch. Entw. Mech. Organ.* **147**, 359-484.
- (1929b). "Untersuchungen an den Staubfaden, Haarzellen und Blattmeristemzellen von *Tradescantia virginica*." *Z. Zellforsch.* **10**, 73-134.
- BELLING, J. (1927). "The attachments of chromosomes at the reduction division in flowering plants." *J. Genet.* **18**, 177-205.
- BERGNER, A. D., CARTLEDGE, J. L. & BLAKESLEE, A. F. (1934). "Chromosome behaviour due to a gene which prevents metaphase pairing in *Datura*." *Cytologia, Tokyo*, **6**, 19-37.
- BHATIA, G. S. (1938). "The cytology of some Indian wheats." *Ann. Bot., Lond., N.S.* **2**, 335-72.
- BLACKBURN, K. B. & HARRISON, J. W. H. (1924). "The origin of a fertile hexaploid form in the *Pimpinellifoliae* \times *villosae* crosses." *Brit. J. exp. Biol.* **1**, 557.

- BRIDGES, C. B. (1921). "Genetical and cytological proof of non-disjunction of the fourth chromosome in *Drosophila melanogaster*." *Proc. nat. Acad. Sci., Wash.*, **7**, 186-92.
- (1923). "Aberrations in chromosome materials." *Eugen., Genet. and the Family*, **1**, 76-7.
- BURNHAM, C. R. (1934). "Chromosome interchange in maize. Reduction of crossing-over and the association of non-homologous parts." *Amer. Nat.* **68**, 91.
- BUXTON, B. H. & NEWTON, W. C. F. (1928). "Hybrids of *Digitalis ambigua* and *Digitalis purpurea*. Their fertility and cytology." *J. Genet.* **19**, 269.
- CANNON, H. G. (1923). "On the nature of the centrosomal force." *J. Genet.* **13**, 47-78.
- CAPINPIN, J. M. (1933). "Studies on the genetics and cytology of triploid *Oenotheras*." *Cytologia, Tokyo*, **4**, 355-426.
- CATCHESIDE, D. G. (1932). "The chromosomes of a new haploid *Oenothera*." *Cytologia, Tokyo*, **4**, 68-113.
- (1933). "Chromosome catenation in some F_1 *Oenothera* hybrids." *J. Genet.* **27**, 45-70.
- (1934). "The chromosomal relationships in the swede and turnip groups of *Brassica*." *Ann. Bot., Lond.*, **48**, 601-33.
- (1935). "X-ray treatment of *Oenothera* chromosomes." *Genetica*, **17**, 313-41.
- (1937). "Secondary pairing in *Brassica oleracea*." *Cytologia, Tokyo*, Fujii Jub. Vol. pp. 366-78.
- CHURCH, G. L. (1929). "Meiotic phenomena in certain Gramineae. II. Paniceae and Andropogoneae." *Bot. Gaz.* **88**, 63-84.
- CLAUSEN, J. (1926). "Genetical and cytological investigations in *Viola tricolor* L. and *V. arvensis* Murr." *Hereditas, Lund*, **8**, 1-156.
- CLAUSEN, R. E. (1927). "Interspecific hybridization in *Nicotiana*. VII. The cytology of hybrids of the synthetic species, *digulata* with its parents, *glutinosa* and *tabacum*." *Univ. Calif. Publ. Bot.* **11**, 177-211.
- CLAUSEN, R. E. & GOODSTEDT, T. H. (1925). "Interspecific hybridization in *Nicotiana*. II. A tetraploid *glutinosa-tabacum* hybrid, an experimental verification of Winge's hypothesis." *Genetics*, **10**, 278-84.
- CLELAND, R. E. (1922). "The reduction division in the pollen-mother cells of *Oenothera franciscana*." *Amer. J. Bot.* **9**, 391-413.
- COLLINS, J. L., HOLLINGSHEAD, L. & AVEEY, P. (1929). "Interspecific hybrids in *Crepis*. III. Constant fertile forms containing chromosomes derived from two species." *Genetics*, **14**, 305-20.
- CREW, F. A. E. & KOLLER, P. C. (1936). "Genetical and cytological studies of the intergeneric hybrid of *Cairina moschata* and *Anas platyrhynca*." *Proc. Roy. Soc. Edinb.* **54**, 210-41.
- DARK, S. O. S. (1936). "Meiosis in diploid and tetraploid *Paeonia* species." *J. Genet.* **32**, 353-72.
- DARLINGTON, C. D. (1930). "Studies in *Prunus*. III." *J. Genet.* **22**, 65-93.
- (1937). *Recent Advances in Cytology*. 2nd ed.
- DARLINGTON, C. D. & MOFFETT, A. A. (1930). "Primary and secondary chromosome balance in *Pyrus*." *J. Genet.* **22**, 129-51.

500 *Cytogenetics of Brassica Hybrids and Species*

- DAVIE, J. H. (1933). "Cytological studies in the Malvaceae and certain related families." *J. Genet.* **28**, 33-67.
- DERMAN, H. (1936). "Fertilization in the Baldwin apple—a triploid variety." *J. Arnold Arbor.* **17**, 106-8.
- DETJEN, L. R. (1927). "Sterility in the common cabbage." *Mem. Hort. Soc. N.Y.* **3**.
- DOBZHANSKY, TH. (1931). "Translocations involving the second and fourth chromosomes of *Drosophila melanogaster*." *Genetics*, **16**, 629-58.
- EAST, E. M. (1933). "The behaviour of a triploid *Nicotiana tabacum* L." *Amer. J. Bot.* **20**, 269-89.
- FARMER & SHOVE (1905). "On the structure and development of somatic and heterotypic chromosomes of *Tradescantia virginica*." *Quart. J. micr. Soc.* **48**, 559-69.
- FOCKE, W. O. (1881). *Die Pflanzen-mischlinge*. Berlin.
- FRANDSEN, H. N. & WINGE, O. (1932). "*Brassica napocampestris*, a new constant amphidiploid species hybrid." *Hereditas, Lund*, **16**, 212.
- FRUWIRTH, C. (1924). *Handbuch der landwirtschaftlichen Pflanzenzüchtung*, **2**. Berlin.
- FUKUSHIMA, E. (1929). "Preliminary report on *Brussico-Raphanus* hybrids." *Proc. imp. Acad. Japan*, **5**, 48-50.
- GAIRDNER, A. E. & DARLINGTON, C. D. (1931). "Ring formation in diploid and polyploid *Campanula persicifolia*." *Genetica*, **13**, 113-50.
- GATES, R. R. (1908). "The chromosomes of *Oenothera*." *Science*, **27**, 193-5.
- (1911). "Pollen formation in *Oenothera gigas*." *Ann. Bot., Lond.*, **25**, 909-40.
- (1912). "Somatic mitosis in *Oenothera*." *Ann. Bot., Lond.*, **26**, 993-1010.
- (1937). "The discovery of the relation between the nucleolus and the chromosomes." *Cytologia, Tokyo*, Fujii Jub. Vol. pp. 977-86.
- (1938). "The species concept in the light of cytology and genetics." *Amer. Nat.* **72**, 340-9.
- GATES, R. R. & CATCHESIDE, D. G. (1932). "Gamolysis of various new *Oenotheras*." *J. Genet.* **26**, 143-78.
- GATES, R. R. & LATTER, J. (1927). "Observations on the pollen development of two species of *Lathraea*." *J. Roy. mic. Soc.* **47**, 209-25.
- GELEI, J. (1921). "Weitere Studien über die Oogenese des *Dendrocoelum lacteum*. II. Die Längskonjugation der Chromosomen." *Arch. Zellforsch.* **16**, 88-169.
- GOODSPEED, T. H. & CLAUSEN, R. E. (1927). "Cytological features of the two F_1 hybrids made with *Nicotiana bigelovii* as a parent." *Univ. Calif. Publ. Bot.* **11**, 117-25.
- GOWEN, J. W. (1928). "Mutation, chromosome non-disjunction and the gene." *Science*, **68**, 211-12.
- GUSTAFSSON, A. (1935). "Studies on the mechanism of parthenogenesis." *Hereditas, Lund*, **21**, 1-112.
- HAGA, T. (1938). "Relationship of Genom to secondary pairing in *Brassica*. (A preliminary note.)" *Jap. J. Genet.* **13**, 277-84.
- HÅKANSSON, A. (1936). "Die Reduktionsteilung in einigen Artbastarden von *Pisum*." *Hereditas, Lund*, **21**, 215-22.
- HEILBORN, O. (1930). "Temperatur und Chromosomen-Konjugation." *Svensk bot. Tidskr.* **24**, 12-25.
- HEITZ, E. (1931). "Nukleolen und Chromosomen in der Gattung *Vicia*." *Planta*, **15**, 495-505.

- HOLLINGSHEAD, L. (1930). "A cytological study of haploid *Crepis capillaris* plants." *Univ. Calif. Publ. agric. Sci.* **6**, 107-34.
- HOWARD, H. W. (1938). "The chromosome number of the swede, *Brassica napus* L." *J. Genet.* **35**, 383-6.
- HUSKINS, C. L. & SMITH, S. G. (1932). "A cytological study of the genus *Sorghum* Pers. The somatic chromosomes." *J. Genet.* **25**, 241-9.
- HUSTED, L. (1936). "An analysis of chromosome structure and behaviour with the aid of X-ray induced rearrangements." *Genetics*, **21**, 537-53.
- IYENGAR, N. K. (1939). "Cytological investigations on the genus *Cicer*. *Ann. Bot. Lond. N.S.* **3**, 271-305.
- JÖRGENSEN, C. A. (1928). "The experimental formation of heteroploid plants in the genus *Solanum*." *J. Genet.* **19**, 133-210.
- KAGAWA, F. (1928). "On the genus crosses between *Triticum* and *Aegilops*." *Jap. J. Bot.* **4**, 2-23.
- KAKIZAKI, Y. (1930). "Studies on the genetics and physiology of self and cross-incompatibility in the common cabbage (*B. oleracea* var. *capitata* L.)." *Jap. J. Bot.* **5**, 134-208.
- KARPECHENKO, G. D. (1922). "The number of chromosomes and the genetic correlation of cultivated Cruciferae." *Bull. Appl. Bot. Pl. Breed.* **13**, 1-14.
- (1927). "Polyploid hybrids of *Raphanus sativus* × *Brassica oleracea* L." *Bull. Appl. Bot. Pl. Breed.* **17**, 305-410.
- (1928). "Polyploid hybrids of *Raphanus sativus* × *Brassica oleracea* L." *Z. indukt. Abstamm.- u. Vererb. Lehre*, **48**, 1-85.
- (1929). "A contribution to the synthesis of a constant hybrid of three species" (English summary). *Proc. U.S.S.R. Congr. Genet.* **2**, 277-94.
- (1937a). "Increasing the crossibility of species by doubling its chromosomes." *Bull. Appl. Bot. Genet. Pl. Breed. Ser. II*, pp. 32-6.
- (1937b). "Experimental production of tetraploid hybrids *Brassica oleracea* L. × *B. carinata* Braun." *Bull. Appl. Bot. Genet. Pl. Breed. Ser. II*, no. 7, pp. 63-68.
- (1937c). "Reciprocal hybrids between *Raphano-Brassica* and tetraploid cabbage." *Bull. Appl. Bot. Genet. Pl. Breed. Ser. II*, no. 7, pp. 451-3.
- KATTERMANN, G. (1933). "Ein Beitrag zur Frage der Dualität der Bestandteile des Bastardkernes." *Planta*, **18**, 751-85.
- KATAYAMA, Y. (1931). "Variation in the number of bivalent chromosomes in the F_1 hybrids between *Triticum durum* and *Aegilops ventricosa*." *Bot. Mag., Tokyo*, **45**, 424-45.
- (1935). "On a chromosomal variant induced by X-ray treatment in *Triticum monococcum*." *Proc. imp. Acad. Japan*, **11**, 110-11.
- KIHARA, H. (1931). "Genomanalyse bei *Triticum* und *Aegilops* II. *Aegilotriticum* und *Aegilops cylindrica*." *Cytologia, Tokyo*, **2**, 106-56.
- KIHARA, H. & LILIENFELD, F. (1934). "Kerneinwanderung und Bildung syndiploide, Pollen-Mutter-Zellen bei dem F_1 Bastard *Triticum aegilopides* × *Aegilops squarrosa*." *Jap. J. Genet.* **10**, 1-28.
- KOLLER, P. C. (1935). "Internal mechanics of chromosomes. IV. Salivary gland chromosomes of *Drosophila*." *Proc. roy. Soc. B*, **118**, 371-97.
- (1936). "Structural hybridity in *Drosophila pseudoobscura*." *J. Genet.* **32**, 79-102.

- KUWADA, Y. (1929). "Chromosome arrangement. "I. Model experiments with floating magnets and some theoretical considerations on the problem." *Mem. Coll. Sci. Kyoto*, Ser. B, **4**, 200-64.
- KUWADA, Y. & SUGIMOTO (1928). "On the staining reactions of chromosomes." *Protoplasma*, **3**, 531-55.
- LAMMERTS, W. E. (1929). "Further studies of the cytology of the back cross progenies of the *paniculata-rustica* hybrid." *Genetics*, **14**, 286-304.
- (1931). "The amphidiploid *rustica* × *paniculata* hybrid, its origin and cytogenetic behaviour." *Genetics*, **16**, 191-211.
- (1932). "An experimentally produced secondary polyploid in the genus *Nicotiana*." *Cytologia, Tokyo*, **4**, 38-45.
- (1934). "On the nature of chromosome association in *Nicotiana tabacum* haploids." *Cytologia, Tokyo*, **6**, 38-50.
- LAWRENCE, W. J. C. (1929). "The genetics and cytology of *Dahlia* species." *J. Genet.* **21**, 125-58.
- (1930). "Incompatibility in polyploids." *Genetica*, **12**, 269-96.
- (1931a). "The genetics and cytology of *Dahlia variabilis*." *J. Genet.* **24**, 257-306.
- (1931b). "The secondary association of chromosomes." *Cytologia, Tokyo*, **2**, 352-84.
- LESLEY, M. M. (1925). "Chromosomal chimaeras in the tomato." *Amer. Nat.* **59**, 570-4.
- LEVAN, A. (1935). "Zytologische Studien an *Allium Schoenoprasum*." *Hereditas, Lund*, **22**, 1-128.
- (1936). "Different results in reciprocal crosses between diploid and triploid *Allium Schoenoprasum*." *Nature, Lond.*, **138**, 508.
- LONGLEY, A. E. (1924). "Cytological studies in the genus *Rubus*." *Amer. J. Bot.* **11**, 249-82.
- (1926). "Triploid citrus." *J. Wash. Acad. Sci.* **16**, 543-45.
- MANTON, I. (1932). "Introduction to general cytology of the Cruciferae." *Ann. Bot., Lond.*, **46**, 509-56.
- MATHER, K. (1935). "Chromosome behaviour in a triploid wheat hybrid." *Z. Zellforsch.* **23**, 117-38.
- (1937). "Notes on the cytology of some *Prunus* species." *Genetica*, **19**, 143-52.
- MAYER, A. M. (1879). "On the morphological laws of configurations formed by magnets floating vertically and subjected to the attraction of superposed magnet." *Philos. Mag.* **7** (cited from Alam, 1936).
- MCCLINTOCK, B. (1931). "Cytological observations of deficiencies involving known genes, translocations and inversions in *Zea Mays*." *Res. Bull. Mo. agric. Exp. Sta.* no. 163.
- (1933). "The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea Mays*." *Z. Zellforsch.* **19**, 191-237.
- (1934). "The relation of a particular chromosomal element to the development of the nucleoli in *Zea Mays*." *Z. Zellforsch.* **21**, 294-328.
- MENSINKAI, S. W. (1939). "Cytological studies in *Allium* species." *J. Genet.* **39**, 1-45.
- METZ, C. W. (1916). "Chromosome studies in *Diptera*. II. The paired association of chromosomes in the diptera and its significance." *J. exp. Zool.* **21**, 213-79.

- MEURMAN, O. (1928). "Cytological studies in genus *Ribes* L." *Hereditas, Lund*, **11**, 289-356.
- (1929). "*Prunus Laurocerasus* L., a species showing high polyploidy." *J. Genet.* **21**, 85-94.
- MOHR, O. L. (1932). "Genetical and cytological proof of somatic elimination of the fourth chromosome in *Drosophila melanogaster*." *Genetics*, **17**, 60-80.
- MOL, W. E. DE (1927). "Nucleolar number in diploid, triploid and aneuploid *Hyacinthus*." *Cellule*, **38**, 1-64.
- MORGAN, T. H. & BRIDGES, C. B. (1919). "The origin of gynandromorphs." *Publ. Carneg. Instn.*, no. 278, pp. 1-122.
- MORGAN, T. H. & STURTEVANT, A. H. (1925). "The genetics of *Drosophila*." *Bibl. Genet.* pp. 1-262.
- MORINAGA, T. (1928). "Preliminary note on interspecific hybridization in *Brassica*." *Proc. imp. Acad. Tokyo*, **4**, 620-2.
- (1929a). "The cytology of F_1 hybrids of *B. napella* and various other species with ten chromosomes." *Cytologia, Tokyo*, **1**, 16-27.
- (1929b). "The cytology of F_1 hybrids *B. cernua* and various other species with ten chromosomes." *Jap. J. Bot.* **4**, 277-89.
- (1929c). "The cytology of F_1 hybrids of *B. cernua* \times *B. napella*." *J. Dep. Agric. Kyushu Univ.* **2**, 199-206.
- (1931). "The cytology of F_1 hybrids of *B. carinata* and some other species with ten chromosomes." *Cytologia, Tokyo*, **3**, 77-83.
- (1933). "The cytology of F_1 hybrids of *B. carinata* \times *B. alboglabra*." *Japan. J. Bot.* **6**, 467-75.
- (1934). "The cytology of F_1 hybrids of *B. juncea* \times *B. nigra*." *Cytologia, Tokyo*, **6**, 62-7.
- MORINAGA, T. & FUKUSHIMA, E. (1933). "Karyological studies on a spontaneous haploid mutant of *Brassica napella*." *Cytologia, Tokyo*, **4**, 457-60.
- MULLER, H. J. (1930). "*Oenothera*-like linkage of chromosomes in *Drosophila*." *J. Genet.* **22**, 335-57.
- MÜNTZING, A. (1930). "Outlines to a genetic monograph on the genus *Galeopsis*." *Hereditas, Lund*, **13**, 185-341.
- (1932). "Cytogenetic investigations on synthetic *Galeopsis Tetrahit*." *Hereditas, Lund*, **16**, 106-54.
- (1933). "Hybrid incompatibility and the origin of polyploidy." *Hereditas, Lund*, **18**, 33-55.
- (1934). "Chromosome fragmentation in a *Crepis* hybrid." *Hereditas, Lund*, **19**, 284-302.
- (1935). "Chromosome behaviour in some *Nicotiana* hybrids." *Hereditas, Lund*, **20**, 251-71.
- NAGAI, K. & SASAOKA, T. (1930). "The number of chromosomes in the cultivated *Brassica*." *Jap. J. Genet.* **5**, 151-8.
- NAITHANI, S. P. (1937). "Chromosome studies in *Hyacinthus orientalis* L. II. Meiotic chromosomes." *Ann. Bot., Lond.*, **1**, 257-75.
- NANDI, H. K. (1936). "The chromosome morphology, secondary association and origin of cultivated rice." *J. Genet.* **33**, 315-36.

- NAVASHIN, M. (1926). "Variabilität des Zellkerns bei *Crepis*-Arten in Bezug auf die Artbildung." *Z. Zellforsch.* **4**, 171-215.
- (1930). "Unbalanced somatic chromosomal variation in *Crepis*." *Univ. Calif. Publ. agric. Sci.* **6**, 95-106.
- (1934). "Chromosome alterations caused by hybridization and their bearing upon genetic problems." *Cytologia, Tokyo*, **5**, 169-203.
- NEWTON, W. C. F. (1924). "Studies on somatic chromosomes. I. Pairing and segmentation in *Galtonia*." *Ann. Bot., Lond.*, **38**, 197-206.
- NEWTON, W. C. F. & DARLINGTON, C. D. (1929). "Meiosis in polyploids." *J. Genet.* **21**, 1-15.
- NEWTON, W. C. F. & PELLEW, C. (1929). "*Primula kewensis* and its derivatives." *J. Genet.* **20**, 405.
- PARTHASARATHY, N. (1938). "Further studies in *Oryza*." *Cytologia, Tokyo*, **9**, 307-18.
- (1938a). "Cytogenetics of some X-ray derivatives in rice (*Oryza sativa* L.)." *J. Genet.* **37**, 1-40.
- (1939). "Cytological studies in Phalarideae." *Ann. Bot., Lond., N.S.* **3**, 43-76.
- PÄTAU, K. (1935). "Chromosomenmorphologie bei *Drosophila melanogaster* und *Drosophila simulans* und ihre genetische Bedeutung." *Naturwissenschaften*, **23**, 537-43.
- PETO, F. H. (1934). "The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*." *J. Genet.* **28**, 113-56.
- POOLE, C. F. (1931). "The interspecific hybrid, *Crepis rubra* × *foetida* and some of its derivatives." *Univ. Calif. Publ. agric. Sci.* **6**, 169-200.
- RAMANUJAM, S. (1937a). "Cytological behaviour of an auto-triploid in rice (*Oryza sativa* L.)." *J. Genet.* **35**, 183-221.
- (1937b). "Cytogenetical behaviour of an interspecific hybrid in *Oryza*." *J. Genet.* **35**, 223-58.
- (1938). "Chromosome studies in the Oryzeae." *Ann. Bot., Lond., N.S.* **2**, 107-25.
- RAMANUJAM, S. & PARTHASARATHY, N. (1935). "An asynaptic mutant in rice." *Proc. Ind. Acad. Sci., Bangalore*, **2**, 80-7.
- RAMIAH, K., PARTHASARATHY, N. & RAMANUJAM, S. (1934). "Chromosome ring in X-rayed rice." *Proc. Ass. Econ. Biol.* pp. 1-4.
- RICHARDSON, M. M. (1936). "Structural hybridity in *Lilium Martagon Album* × *L. Hansonii*." *J. Genet.* **32**, 411-49.
- RICHHARIA, R. H. (1937a). "Cytological investigations of *Raphanus sativus* × *Brassica oleracea* and their F_1 and F_2 hybrids." *J. Genet.* **34**, 19-44.
- (1937b). "Cytological investigations of ten chromosome species of *Brassica* and their F_1 hybrids." *J. Genet.* **34**, 45-55.
- ROSENBERG, O. (1917). "Die Reduktionsteilung und ihre Degeneration in *Hieracium*." *Svensk bot. Tidskr.* **11**, 145-206.
- (1927). "Die semiheterotypische Teilung und ihre Bedeutung für die Entstehung verdoppelter Chromosomenzahlen." *Hereditas, Lund*, **8**, 305-38.
- RUTTLE, M. L. (1928). "Chromosome numbers and morphology in *Nicotiana*. II. Diploidy and partial diploidy in root tips of *Tabacum* haploids." *Univ. Calif. Publ. Bot.* **11**, 213-32.

- SAKAI, K. I. (1935). "Chromosome studies in *Oryza sativa*." *Jap. J. Genet.* **11**, 145-56.
- SANSOME, E. R. (1929). "A chromosome ring in *Pisum*." *Nature, Lond.*, **124**, 578.
- (1932). "Segmental interchange in *Pisum*." *Cytologia, Tokyo*, **3**, 200-19.
- SAPEHIN, L. A. (1933). "The genes of the reduction division." *Bull. Appl. Bot. Genet. Pl. Breed. Ser. II*, no. 5, pp. 43-75.
- SASAOKA, T. (1930). "Karyological observations in different interspecific hybrids of *Brassica*." *Jap. J. Genet.* **6**, 20-32.
- SATO, D. (1937). "Analysis of karyotypes in Aloinae with special reference to SAT-chromosomes." *Cytologia, Fujii Vol.*, 80-95.
- SAX, K. (1930). "Chromosome structure and mechanism of crossing-over." *J. Arnold Arbor.* **11**, 193-220.
- (1931). "Chromosome ring formation in *Rhoeo discolor*." *Cytologia, Tokyo*, **2**, 1-26.
- (1937). "Chromosome inversions in *Paeonia suffruticosa*." *Cytologia, Tokyo, Fujii Jub. Vol.* pp. 108-4.
- SCHAVINSKAYA, S. A. (1937). "Tetraploid cabbage obtained by means of regeneration." *Bull. Appl. Bot. Genet. Pl. Breed. Ser. II*, no. 7, pp. 32-6.
- SEMMENS, C. S. (1937). "A substitute for osmic acid." *The Microscope*, **1**, 29-31.
- SEMMENS, C. S. & BHADURI, P. N. (1939). "The technique for differential staining of nucleoli and chromosomes." *Stain Techn.* **14**, 1-5.
- SHIMOTOMAI, N. (1925). "A karyological study of *Brassica*." *Bot. Mag., Tokyo*, **39**, 122-7.
- SINAKATA, E. (1927). "Geno-systematical investigations of cultivated *Brassica*." *Bull. Appl. Bot. Pl. Breed.* **17**, 1-166.
- SKOVSTED, A. (1933). "Cytological studies in Cotton. I. The mitosis and meiosis in diploid and triploid Asiatic cotton." *Ann. Bot., Lond.*, **47**, 227-51.
- (1937). "Cytological studies in cotton. IV. Chromosome conjugation in interspecific hybrids." *J. Genet.* **34**, 97-134.
- SMITH, S. G. (1935). "Chromosome fragmentation produced by crossing-over in *Trillium erectum* L." *J. Genet.* **30**, 227-32.
- SRINATH, K. V. (1939). "Morphological and cytological studies in the genus *Calceolaria* L. IV." *Zeits. f. Abst. v. Vererb. Lehre*, **77**, 104-34.
- STADLER, L. J. (1932). "On the genetic nature of induced mutations in plants." *Proc. 6th Internat. Congr. Genet.* pp. 274-94.
- STOW, I. (1926). "A cytological study of pollen-sterility in *Solanum tuberosum*." *Proc. imp. Acad. Japan*, **2**, 426-30.
- (1927). "A cytological study of pollen-sterility in *Solanum tuberosum*." *Jap. J. Bot.* **3**, 217-38.
- STRASBURGER (1905). Cited from Gates (1912).
- STURTEVANT, A. H. (1926). "A cross-over reducer in *Drosophila melanogaster*, due to inversion of a section of the third chromosome." *Biol. Zbl.* **46**, 697-702.
- STURTEVANT, A. H. & DOBZHANSKY, TH. (1936). "Inversions in the third chromosome of wild races of *Drosophila pseudoobscura* and their use in the history of species." *Proc. nat. Acad. Sci., Wash.*, **22**, 448-50.
- SWEZY, O. (1937). "Alterations in somatic chromosomes in *Crepis*." *Cytologia, Tokyo, Fujii Jub. Vol.* pp. 149-55.

- TÄCKHOLM, G. (1922). "Zytologische Studien über die Gattung *Rosa*." *Acta Hort. berg.* **7**, 97-380.
- TSCHERMAK, E. & BLEIER, H. (1926). "Über fruchtbare *Aegilops*-Weizen-Bastarde." *Ber. dtsh. bot. Ges.* **44**, 110-32.
- U, N. (1935). "Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization." *Jap. J. Bot.* **7**, 388-452.
- UPCOTT, M. (1937). "Spontaneous chromosome changes in pollen grains." *Nature, Lond.*, **139**, 153.
- (1937a). "The genetic structure of *Tulipa*." *J. Genet.* **34**, 339-97.
- WATKINS, A. E. (1932). "Hybrid sterility and incompatibility." *J. Genet.* **25**, 125-62.
- WHYTE, R. O. (1930). "Sterility and floral abnormality in the tetraploid *Saxifraga potternensis*." *J. Genet.* **23**, 93-121.
- WINGE, O. (1917). "The chromosomes, their numbers and general importance." *C.R. trav. Lab. Carlsberg*, **13**, 131.
- WRIGHT, F. R. E. (1936). "The Lundy *Brassica* with some additions." *J. Bot.* pp. 1-8.
- YARNELL, S. H. (1931). "A study of certain polyploid and aneuploid forms of *Fragaria*." *Genetics*, **16**, 455-89.

EXPLANATION OF PLATES XVI-XX

All drawings were made at bench level with the aid of a Camera lucida using Beck's 2 mm. apochromatic objective N.A. 1.2 in conjunction with Zeiss eye piece K. 30 X and Beck's X 25 and X 17, which gave approximate magnifications of 4300, 3350 and 2450 diameters, respectively. Figs. 151 and 152 were drawn with the eye piece 17, fig. 85 with 25 and all the rest with 30. All drawings have been reduced to one-half in reproduction.

PLATE XVI

- Figs. 1-4. *Brassica juncea*. Diakinesis. Fig. 1, 18_{II} ; Fig. 2, $1(4) + 16_{II}$ (three bivalents are attached to the nucleolus); Fig. 3, $15_{II} + 1_{III} + 3_I$; Fig. 4, $1(4) + 12_{II}$ (the nucleus is cut).
- Fig. 5. *B. juncea*. Metaphase I showing 18_{II} (polar view).
- Fig. 6. *B. juncea*. Metaphase I showing $17_{II} + 2_I$ (polar view).
- Fig. 7. *B. juncea*. Metaphase I (side view) showing a chain of three chromosomes, and three univalents lying off the plate (not all the chromosomes are drawn).
- Fig. 8. *B. juncea*. Metaphase I (polar view) showing $1_{IV} + 16_{II}$.
- Fig. 9. *B. juncea*. Anaphase I showing the splitting of two lagging univalents.
- Fig. 10. *B. juncea*. Telophase I showing the split halves of a univalent lying in the cytoplasm.
- Fig. 11. *B. campestris* var. *sarson*. Diakinesis showing ten bivalents.
- Fig. 12. *B. campestris* var. *sarson*. Metaphase I (polar view) showing 10_{II} .
- Fig. 13. *B. juncea* \times *B. campestris* (F_1). Diakinesis showing $10_{II} + 8_I$.
- Fig. 14. *B. juncea* \times *B. campestris* (F_1). Metaphase I (polar view) showing $10_{II} + 8_I$.
- Fig. 15. *B. juncea* \times *B. campestris* (F_1). Metaphase I (polar view) showing sixteen chromosomes consisting of $1_{IV} + 9_{II} + 6_I$.
- Fig. 16. *B. juncea* \times *B. campestris* (F_1). Metaphase I (side view) showing 10_{II} forming a plate and 8_I scattered on the spindle.
- Fig. 17. *B. juncea* \times *B. campestris* (F_1). Metaphase I (side view) showing 10_{II} and four univalents lying on the equator. The other four univalents are lying off the plate.
- Fig. 18. *B. juncea* \times *B. campestris* (F_1). Early anaphase I showing the disjoining of the components of bivalent chromosomes.

- Fig. 19. *B. juncea* \times *B. campestris* (F_1). Anaphase I. The disjoined members of the bivalent chromosomes have reached the pole and the equational split in some univalent is becoming apparent.
- Fig. 20. *B. juncea* \times *B. campestris* (F_1). Anaphase I showing the division of univalents at the equator. An undivided univalent is passing intact to the lower pole.
- Fig. 21. *B. juncea* \times *B. campestris* (F_1). Anaphase I showing the split halves of a univalent moving to the same pole.
- Fig. 22. *B. juncea* \times *B. campestris* (F_1). Metaphase II plate with nearly twenty-eight chromosomes formed as a result of division I restitution nucleus.
- Fig. 23. *B. juncea* \times *B. campestris* (F_1). Metaphase II plates with thirteen and sixteen chromosomes, respectively. Two univalents are lying in the cytoplasm.
- Fig. 24. *B. juncea* \times *B. campestris* (F_1). Metaphase II plates with fifteen and seventeen chromosomes, respectively.
- Fig. 25. *B. juncea* \times *B. campestris* (F_1). Telophase I showing that all the divided or undivided univalents have been included in the daughter nuclei.
- Fig. 26. *B. juncea* \times *B. campestris* (F_1). Telophase I showing lagging of univalents.
- Figs. 27 and 29. *B. juncea* \times *B. campestris* (F_1). Anaphase II showing lagging of univalents.
- Fig. 28. *B. juncea* \times *B. campestris* (F_1). Anaphase II showing the formation of a chromatin bridge.
- Fig. 30. *B. juncea* \times *B. campestris* (F_1). Telophase II showing stray chromosomes in the cytoplasm.
- Fig. 31. *B. Tournefortii*. Diakinesis showing 10_{II}, one bivalent is attached to the nucleolus.
- Fig. 32. *B. Tournefortii*. Diakinesis showing 9_{II} and 2_I.
- Fig. 33. *B. Tournefortii*. Metaphase I (polar view) with ten bivalents.
- Fig. 34. *B. Tournefortii*. Anaphase I showing distribution of ten chromosomes to each pole.
- Fig. 35. *B. trilocularis*. Diakinesis showing 10_{II}, one attached to the nucleolus.

PLATE XVII

- Fig. 36. *B. trilocularis*. Metaphase I (polar view) showing 10_{II}.
- Figs. 37-41. *B. Tournefortii* \times *B. trilocularis* (F_1). Diakinesis. Fig. 37, 20_I, one showing an apparent satellite; Fig. 38, 1_{III} + 18_I; Fig. 39, 2_{II} + 16_I; Fig. 40, 3_{II} + 14_I; Fig. 41, 1_{III} + 17_I.
- Fig. 42. *B. Tournefortii* \times *B. trilocularis* (F_1). Diakinesis showing 1_{IV} + 16_I.
- Fig. 43. *B. Tournefortii* \times *B. trilocularis* (F_1). Metaphase I (polar view) with seventeen chromosomes consisting of 3_{II} + 14_I. Excepting two univalents all the chromosomes are located on the equator.
- Fig. 44. *B. Tournefortii* \times *B. trilocularis* (F_1). Metaphase I (side view) with 20_I.
- Fig. 45. *B. Tournefortii* \times *B. trilocularis* (F_1). Metaphase I (side view) with two bivalents lying on the equator and sixteen univalents scattered on the spindle.
- Fig. 46. *B. Tournefortii* \times *B. trilocularis* (F_1). Metaphase I (side view) showing a trivalent and one univalent at the equator. The other sixteen univalents are located at the other pole.
- Fig. 47. *B. Tournefortii* \times *B. trilocularis* (F_1). Metaphase I (side view) showing 1_{III} + 2_{II} + 13_I.
- Fig. 48. *B. Tournefortii* \times *B. trilocularis* (F_1). Metaphase I (side view) showing 1_{III} + 2_{II} + 1: ten univalents are arranged on the equator and three are lying off the plate.
- Fig. 49. *B. Tournefortii* \times *B. trilocularis* (F_1). Profile metaphase I showing 1_{IV} + 3_{II} + 10_I.
- Fig. 50. *B. Tournefortii* \times *B. trilocularis* (F_1). Profile metaphase II with twenty-two chromosomes resulting from restitution nucleus.
- Figs. 51-60. *B. Tournefortii* \times *B. trilocularis* (F_1). Pollen mother cells showing metaphase II plates with varying numbers of chromosomes. Fig. 51, 10 + 10; Fig. 52, 9 + 15; Fig. 53, 8 + 12; Fig. 54, 12 + 10; Fig. 55, 11 + 8 and split halves of a univalent passing to the same pole; Fig. 56, 10 + 6 and four univalents in the cytoplasm; Fig. 57, 7 + 13; Fig. 58, 10 + 11 and one univalent in the cytoplasm; Fig. 59, 12 + 12; Fig. 60, 8 + 13 and one univalent in the cytoplasm.

508 *Cytogenetics of Brassica Hybrids and Species*

- Fig. 61. *B. Tournefortii* × *B. trilocularis* (F_1). Anaphase I showing a trivalent passing intact to the upper pole.
 Fig. 62. *B. Tournefortii* × *B. trilocularis* (F_1). Anaphase I showing a univalent bridge.
 Fig. 63. *B. Tournefortii* × *B. trilocularis* (F_1). Division I restitution nucleus including nineteen chromosomes. One chromosome has not been included in the nucleus.
 Fig. 64. *B. Tournefortii* × *B. trilocularis* (F_1). Anaphase II showing a supernumerary spindle.

PLATE XVIII

- Figs. 65-67. *B. Tournefortii* × *B. trilocularis* (F_1). Anaphase II showing lagging chromosomes and double division of univalents.
 Fig. 68. *B. Tournefortii* × *B. trilocularis* (F_1). Pollen mother cell showing the fusion of two homotypic spindles, and a number of lagging chromosomes.
 Fig. 69. *B. Tournefortii* × *B. trilocularis* (F_1). Pollen mother cell showing the union of two division II anaphasic groups.
 Fig. 70. *B. Tournefortii* × *B. trilocularis* (F_1). Pollen mother cell in telophase II with stray chromosomes in the cytoplasm.
 Fig. 71. *B. Tournefortii* × *B. trilocularis* (F_1). Pollen mother cell showing the formation of micronuclei.
 Fig. 72. *B. rapa*. Diakinesis showing ten bivalents, one attached to the nucleolus.
 Fig. 73. *B. rapa*. Metaphase I with 10_{II}.
 Fig. 74. *B. rapa* × *B. trilocularis* (F_1). Diakinesis showing 10_{II}.
 Fig. 75. *B. rapa* × *B. trilocularis* (F_1). Metaphase I showing 10_{II}.
 Fig. 76. *B. rapa* × *B. trilocularis* (F_1). Anaphase I showing distribution of eleven and nine chromosomes to opposite poles (non-disjunction).
 Fig. 77. *B. rapa* × *B. trilocularis* (F_1). Telophase I showing five persisting nucleolar-like bodies in the cytoplasm.
 Fig. 78. *B. rapa* × *B. trilocularis* (F_1). Telophase II showing six persisting nucleolar-like bodies in the cytoplasm.
 Fig. 79. *B. nigra*. Somatic plate with sixteen chromosomes, three of which are satellited and one shows only a filament without a satellite.
 Fig. 80. *B. oleracea*. Somatic plate with eighteen chromosomes, two of which are satellited.
 Figs. 81-84. Somatic plates in *B. rapa*, *B. campestris* var. *sarson*, *B. trilocularis*, *B. Tournefortii*, respectively. Each has twenty chromosomes, two of which are satellited.
 Fig. 85. *B. juncea*. Somatic plate with thirty-six chromosomes, six of which are satellited.
 Fig. 86. Somatic plate in *B. rugosa* with thirty-eight chromosomes and probably four satellites.

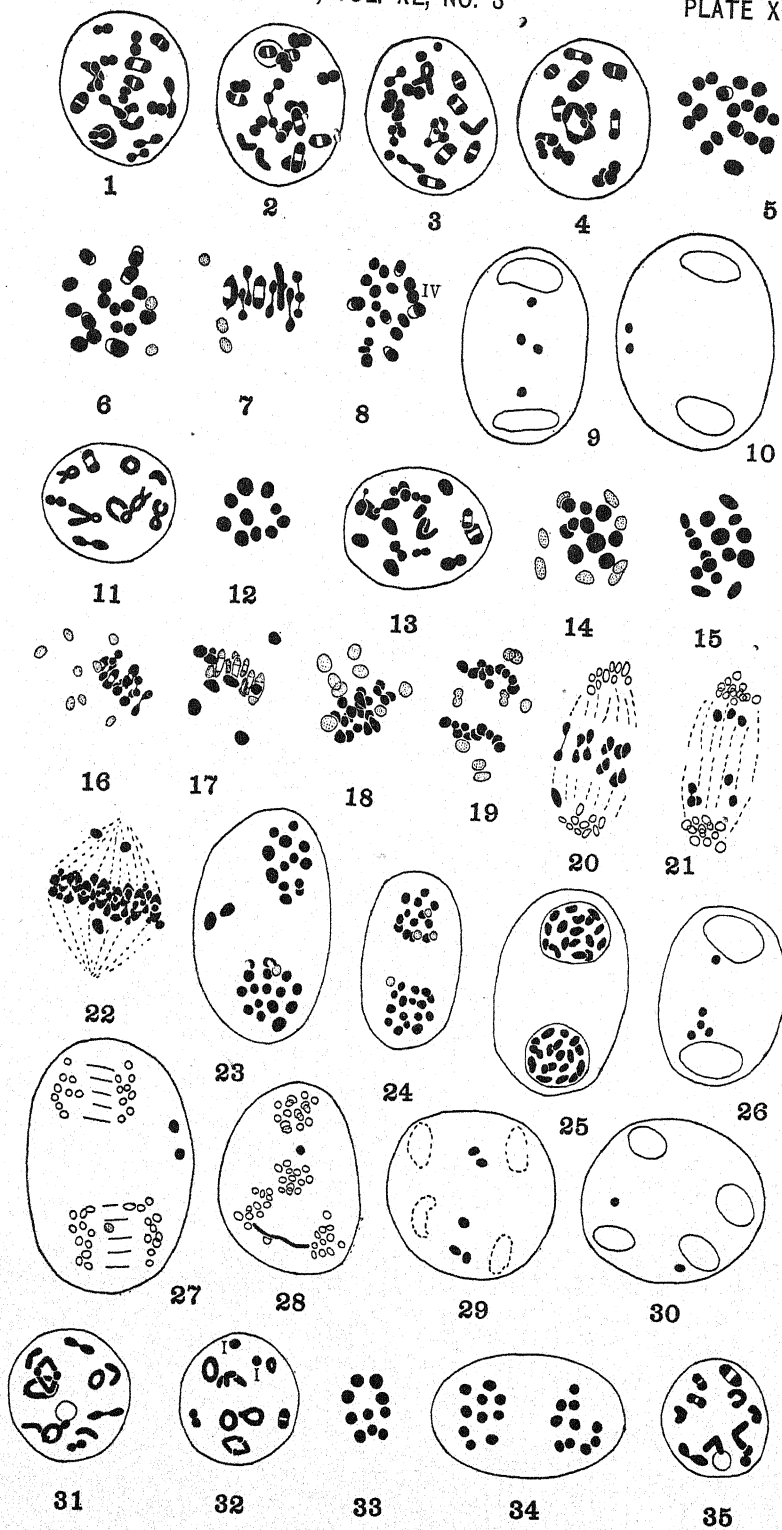
PLATE XIX

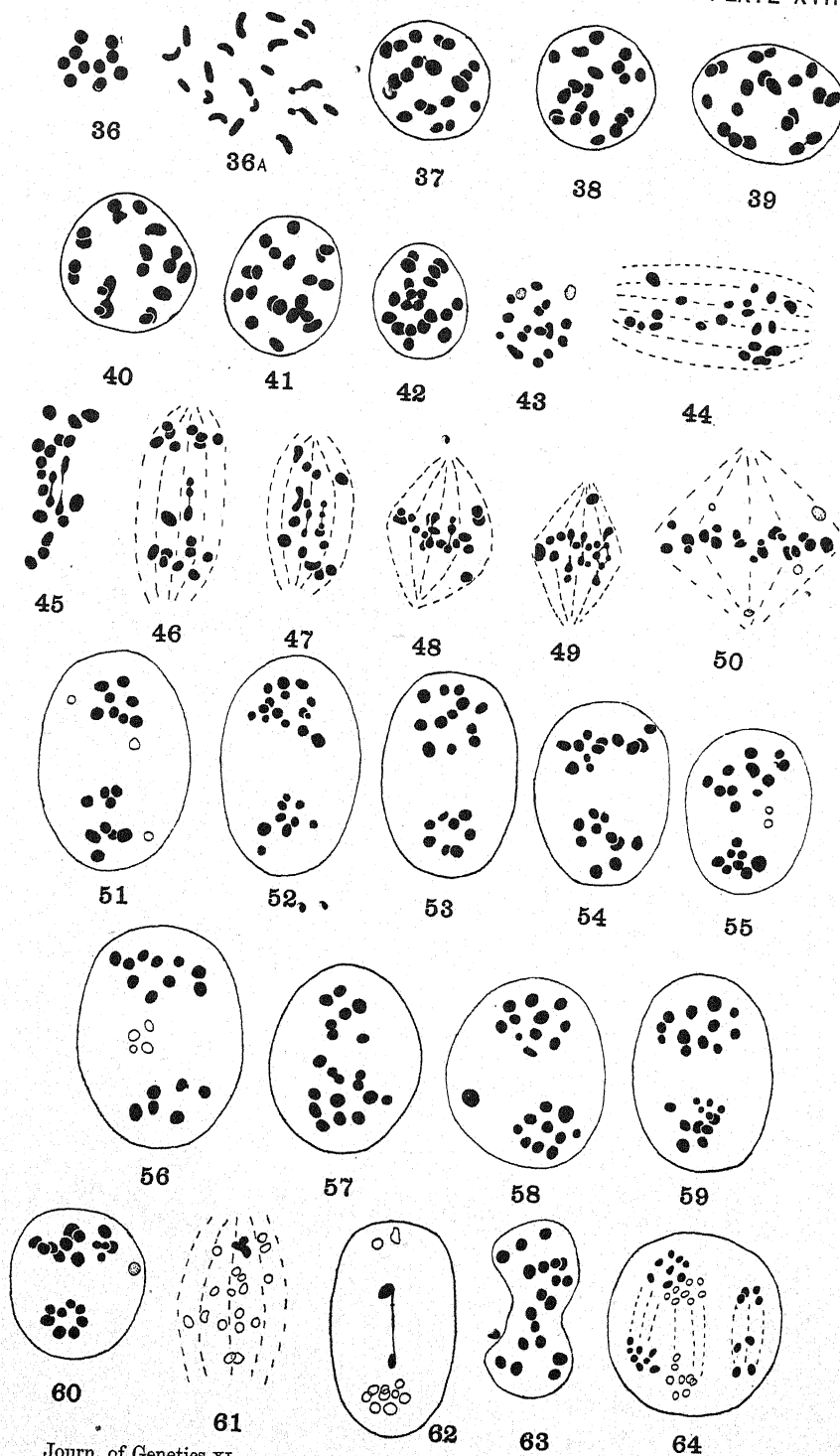
- Fig. 87. Somatic plate in *B. napus* with thirty-eight chromosomes and probably four satellites.
 Fig. 88. *B. campestris*. Somatic plate showing the grouping of chromosomes on the periphery of the plate.
 Fig. 89. *B. Tournefortii*. Somatic plate with twenty-four chromosomes, the genome of four being split.
 Fig. 90. *B. trilocularis*. Somatic telophase showing a chromatin bridge and a fragment.
 Fig. 91. *B. nigra*. Somatic telophase with four nucleoli in each pole.
 Fig. 92. *B. oleracea*. Somatic telophase with two nucleoli in each pole.
 Fig. 93. *B. rapa*. Somatic telophase showing two nucleoli in each daughter nucleus.
 Fig. 94. *B. juncea*. Resting nucleus with six nucleoli.
 Fig. 95. *B. rugosa*. Somatic telophase showing four nucleoli in each daughter nucleus.
 Fig. 96. *B. monensis*. Late diplotene showing twelve bivalents, one of which is attached to the nucleolus.
 Fig. 97. *B. monensis*. Various types of bivalents at diplotene drawn from different nuclei.

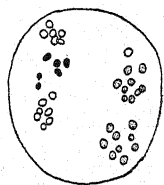
- Figs. 98 and 99. *B. monensis*. Diakinesis. Fig. 98 shows 12_{II} , while Fig. 99 has a ring of four and ten bivalents.
- Figs. 100 and 101. *B. monensis*. Metaphase I showing forms of arrangement. Fig. 100 has three bivalents inside and nine outside. Fig. 101 has four inside and eight on the periphery.
- Figs. 102 and 103. *B. monensis*. Metaphase I showing eleven bivalents and two univalents. Fig. 102, profile; Fig. 103, polar view.
- Fig. 104. *B. monensis*. Metaphase II showing distribution of thirteen and ten chromosomes.
- Fig. 105. *B. monensis*. Anaphase I showing a lagging bivalent.
- Figs. 106–111. *B. monensis*. Metaphase II showing various degrees of secondary association. Fig. 106, $1(2)+10(1)$; Fig. 107, $2(2)+8(1)$; Fig. 108, $3(2)+6(1)$; Fig. 109, $5(2)+2(1)$; Fig. 110, $1(3)+9(1)$; Fig. 111, $1(3)+1(2)+7(1)$.
- Figs. 112–115. *B. monensis*. Metaphase II plates showing various degrees of secondary association of chromosomes. Fig. 112, $1(3)+2(2)+5(1)$; Fig. 113, $1(3)+3(2)+3(1)$; Fig. 114, $1(4)+2(2)+4(1)$; Fig. 115, maximum association $1(4)+1(3)+2(2)+1(1)$.
- Figs. 116–120. *B. Wrightii*. Diakinesis. Fig. 116, 12_{II} ; Fig. 117, $2_{IV}+8_{II}$; Fig. 118, $1_{VI}+7_{II}$; Fig. 119, $1_{VIII}+8_{II}$; Fig. 120, 1_V+7_{II} .

PLATE XX

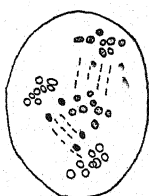
- Fig. 121. *B. Wrightii*. $1_{XX}+2_{II}$.
- Fig. 122 A–L. *B. Wrightii*. Showing various multivalent configurations from different nuclei. A, D, F, G and I quadrivalents; B and K sexivalents; E and J octovalents; H and L decavalent; C, a K-shaped configuration formed by ten bivalents.
- Fig. 123. *B. Wrightii*. Anaphase I showing a dicentric chromatin bridge.
- Fig. 124. *B. sinapistrum*. Somatic plate with eighteen chromosomes.
- Figs. 125 and 126. *B. sinapistrum*. Diakinesis. Fig. 125, 9_{II} ; Fig. 126, $1_{IV}+7_{II}$.
- Fig. 127. *B. sinapistrum*. Metaphase I (side view) with 9_{II} . (The bivalents have been separated out in drawing.)
- Fig. 128. *B. sinapistrum*. Metaphase I (side view) with $8_{II}+2_I$.
- Fig. 129. *B. sinapistrum*. Metaphase II showing distribution of ten and eight chromosomes (non-disjunction).
- Figs. 130–136. *B. sinapistrum*. Metaphase II plates showing various degrees of secondary association. Fig. 130, 9_I ; Fig. 131, $1(2)+7(1)$; Fig. 132, $2(2)+5(1)$; Fig. 133, $3(2)+3(1)$; Fig. 134, $1(3)+6(1)$; Fig. 135, $1(3)+1(2)+4(1)$; Fig. 136, $4(2)+1(1)$ maximum association.
- Figs. 137–140. *B. nigra*. Diakinesis. Fig. 137, 8_{II} ; Fig. 138, $2_{IV}+4_{II}$; Fig. 139, $1_{IV}+6_{II}$. Two bivalents are attached to the nucleolus; Fig. 140, $1_{VIII}+3_{II}$ one bivalent is extruded from the nucleus.
- Figs. 141–143. *B. nigra*. Metaphase I. Fig. 141 (side view) with 8_{II} (the bivalents have been separated out in drawing); Fig. 142, $1_{IV}+6_{II}$; Fig. 143, $1_{VI}+5_{II}$.
- Figs. 144–149. *B. nigra*. Metaphase I plates showing various degrees of secondary association. Fig. 144, $2(2)+4(1)$; Fig. 145, $3(2)+2(1)$; Fig. 146, $3(2)+2(1)$ (metaphase II); Fig. 147, $1(3)+1(2)+3(1)$; Fig. 148, $1(5)+1(2)+1(1)$; Fig. 149, $1(7)+1(1)$.
- Figs. 150 and 151. *B. nigra*. Pollen mother cells showing cytomixis. Fig. 150, metaphase I; Fig. 151, telophase I.
- Fig. 152. *B. nigra*. Metaphase I (profile) showing non-orientation of two bivalents.
- Fig. 153. *B. cheiranthus*. Polar metaphase I with 24_{II} secondarily paired into $1(4)+2(3)+5(2)+4(1)$.



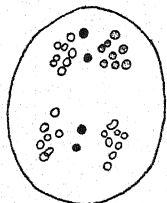




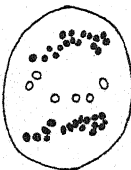
65



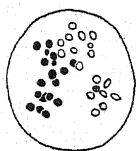
66



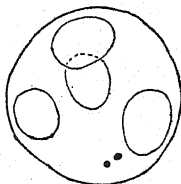
67



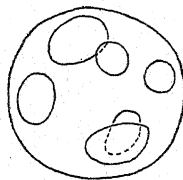
68



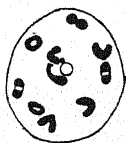
69



70



71



72



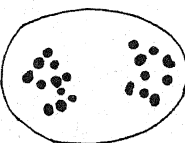
73



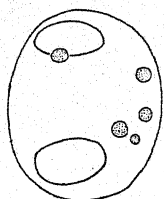
74



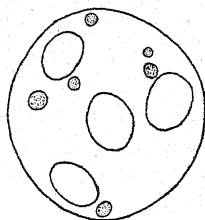
75



76



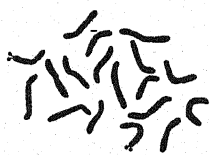
77 ..



78



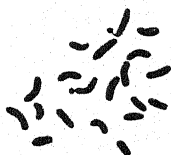
79



80



81



82



83



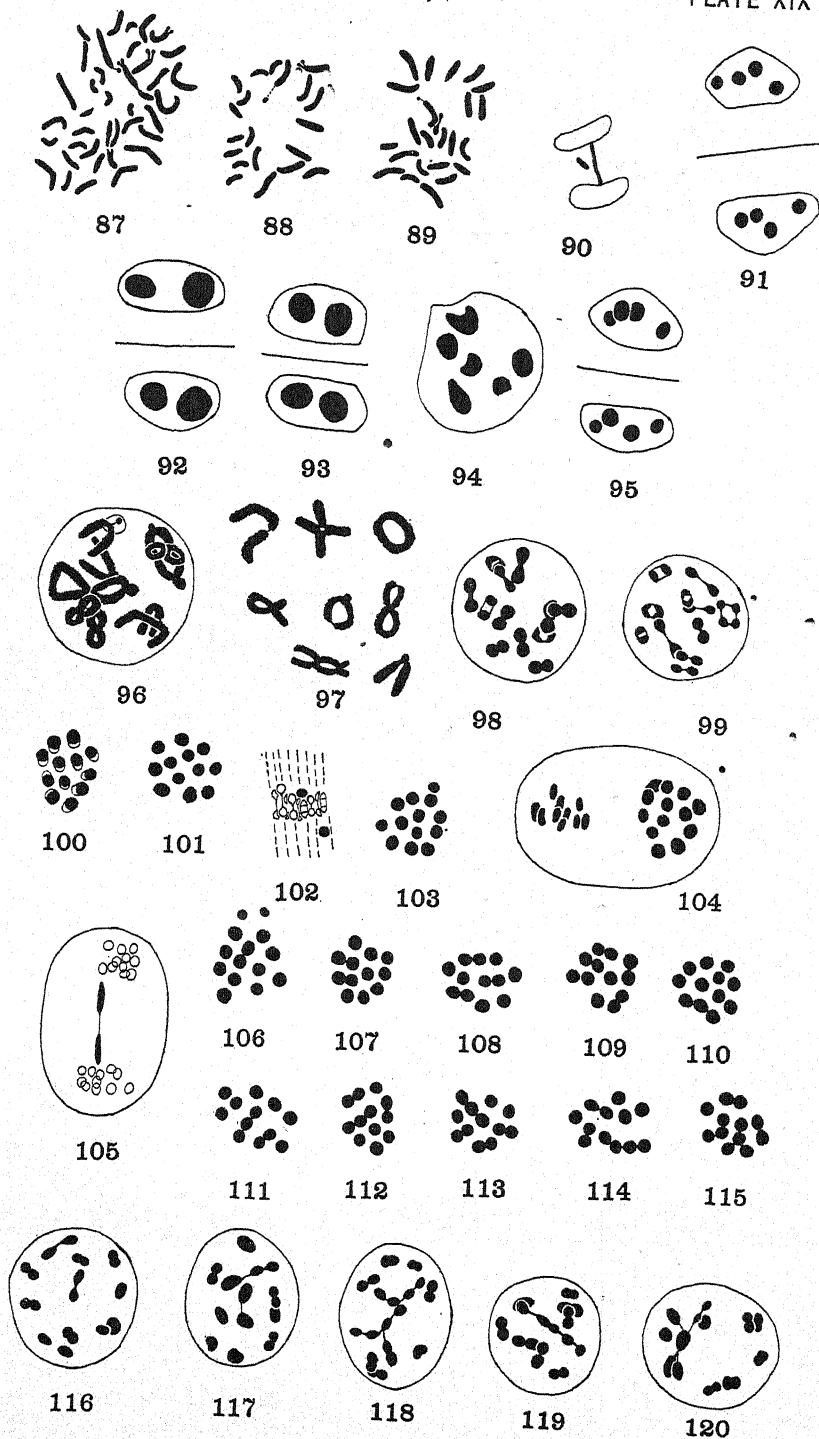
84

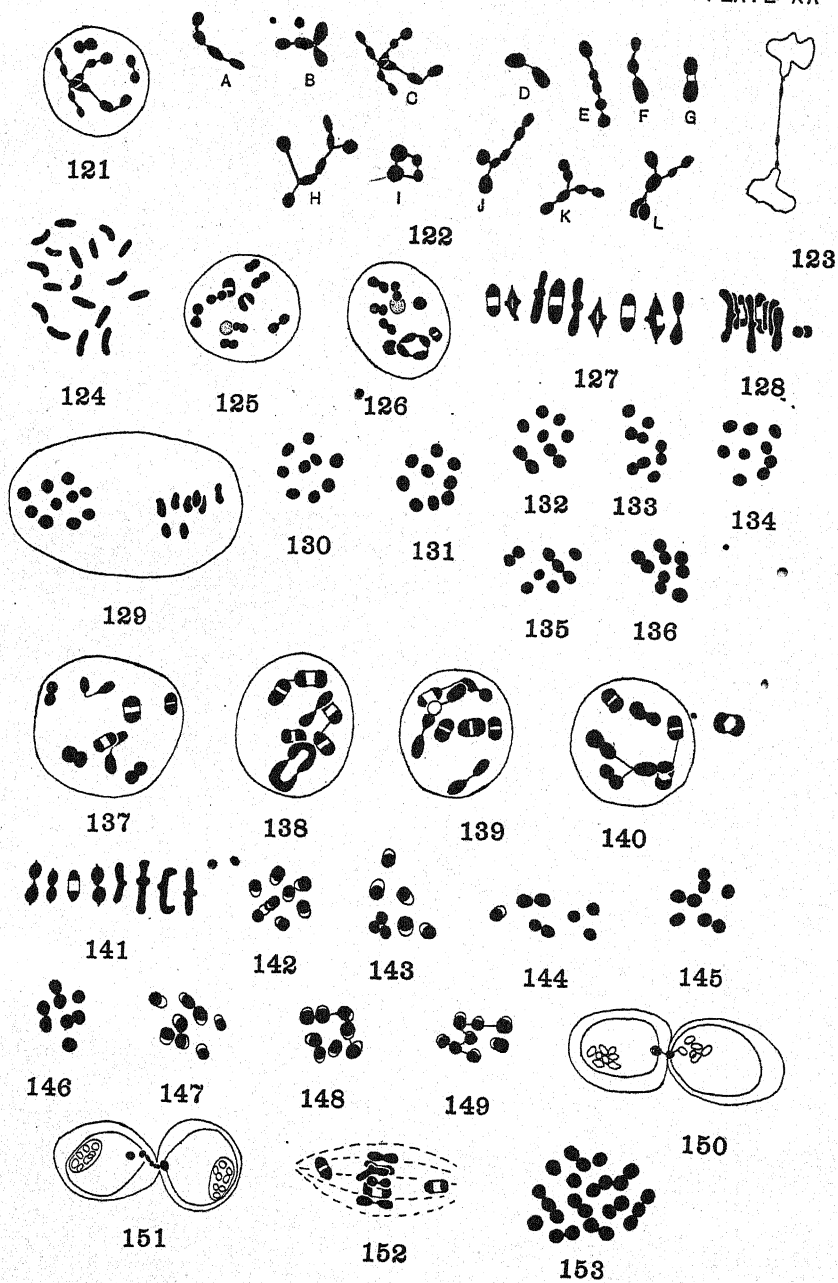


85



86





3
CAMBRIDGE: PRINTED BY
W. LEWIS, M.A.
AT THE UNIVERSITY PRESS